COBALT CHLORIDE TREATMENT AND IRON METABOLISM IN IMMATURE MICE

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Abstract. Although cobalt is an essential trace element, it is toxic in high concentrations. Long-term exposure to cobalt chloride ($CoCl_2$) significantly increases Co(II) ions in blood serum, spleen and liver of treated immature mice compared to controls and induces changes in the iron (Fe) content. Spleen and liver show different sensitivity to Co(II) administration but increase iron storage. The three experimental groups of immature mice – day 18-, 25- and 30, used in the experimental design, show different sensitivity to the metal. This suggests that the stage of development is also an important marker that should be considered.

Key words: cobalt chloride, iron metabolism, immature mice, liver, spleen

INTRODUCTION

Cobalt(II) is widely used as a nutritional supplement, preservative, in drinks, cosmetics, medical devices, as therapeutic agent for treating different diseases, etc. 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. 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]. The concentration of Co(II) is also increased in whole blood, serum and urine [13, 14]. Young animals (rats and guinea pigs) have 3- to 15-fold greater absorption than adult animals. Water-soluble cobalt compounds exhibit greater absorption than non-water-soluble forms but absorption is species dependent [17]. Co treatment is shown to improve tissue adaptation to hypoxia, enhances physical endurance performance and ameliorates mountain sickness [14]. Administration of a given element to animals can affect the metabolism and tissue distribution of other metals by altering the function or content of specific metal-binding proteins, or by competing for similar binding sites within cells [13]. Co competes with iron for globin moiety and binds irreversibly in red blood cells [14]. Gleason et al. show that Co as a hypoxia-mimicking agent affects the activity of iron-containing proteins, thus altering different biochemical pathways [5]. Long-term exposure to cobalt chloride ($CoCl_2$) may possibly trigger a cascade of biochemical reactions, which will induce changes in signal transduction, protein biosynthesis and other biological processes. There is lack of data for the affect of long-term Co(II) treatment on other trace elements' metabolism and tissue distribution which could contribute to the toxic effect of cobalt ions on one hand, and affect animal development, on the other.

Iron (Fe) is an essential element for cellular metabolism, DNA synthesis, aerobic respiration and key metabolic reactions. Fe homeostasis including iron-loading, transport and storage must be delicately regulated, as iron loading leads to free radical damage by the Fenton reaction [4, 12]. It is stored mainly in the spleen and liver.

The aim of the present study is to investigate the effect of long-term exposure to $CoCl_2$ on iron metabolism in immature mice.

MATERIALS AND METHODS

Experimental design

Pregnant ICR mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H_2O$) treatment at daily doses of 75 mg/kg or 125 mg/kg. The compound was dissolved and obtained from drinking tap water. 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 to ensure that all experimental animals obtained the required dose $CoCl_2$. They were weighed weekly and the experimental cobalt concentration was adjusted accordingly. The newborn pups were sacrificed on days 18, 25 and 30 which corresponded to different stages of development. Blood serum was collected, stored at -20˚C until further analysis. Spleen and liver were excised and also prepared for analysis. 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.

Biochemical assays

Cobalt and iron concentrations were determined in blood serum using electro-thermal atomic absorption spectrometry (ET-AAS) using Zeeman Perkin Elmer 3030 instrument, HGA 600, pyrolitic coated graphite tube as atomizer. Their concentrations in the spleen and liver of the experimental mice were measured 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 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

Data show that cobalt is transferred from food into the mother's milk [10, 16], therefore the newborn pups were exposed to Co(II) through their nursing period.

Reduction in food or water consumption was not observed and all experimental animals survived through the experiment. Our previous studies had not shown any sex differences in the hematological parameters, therefore the experimental groups consisted of both male and female mice from the same litter (n=3).

Preliminary results show that treatment with $CoCl_2$ induced significant accumulation of the metal ions in blood serum compared to the untreated control (Fig.1). A dose-dependent effect was observed having higher Co(II) concentrations in the serum samples of mice, treated with the high daily dose (125 mg/kg). This result corresponds to our findings for induced hematological changes in Co-treated mice [6].

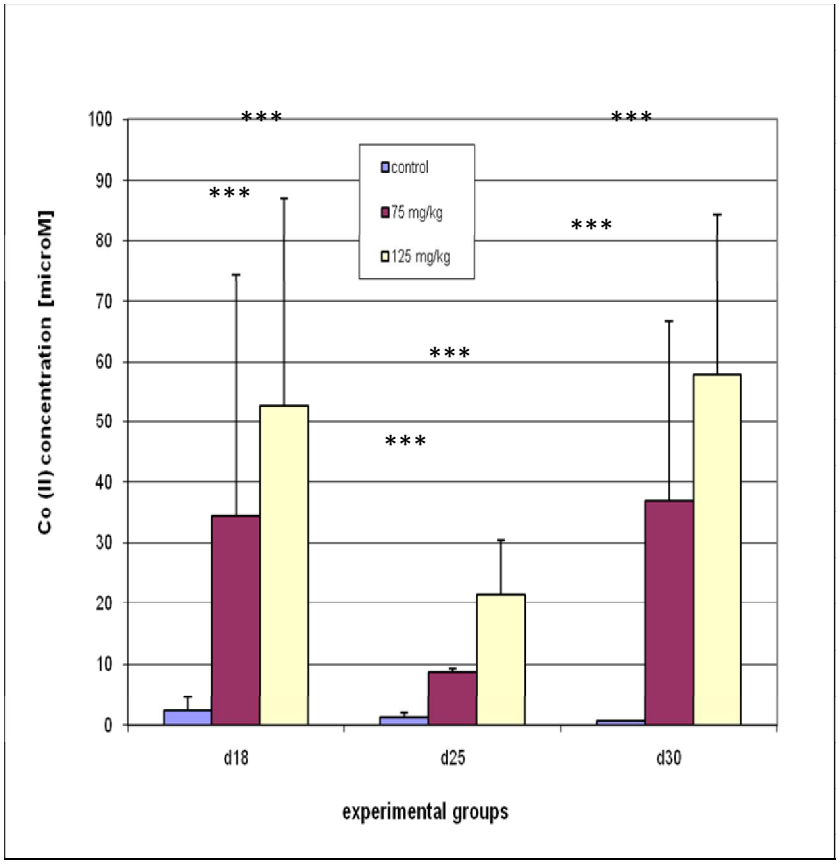

Fig.1. Co(II) concentration in blood serum. Each column represents mean±SD, *n* = 3. Triple asterisk (***) represents statistical difference ($p<0.001$).

The cobalt (II) ions measured in the control samples are probably due to cobalt-containing supplements in the obtained commercially bought food chow.

The accumulation of Co(II) induced changes in the Fe concentration (Fig.2). In blood serum of day 18 mice less Fe was measured in the Co-treated animals compared to the controls. The lowest Fe concentration in day 18 mice was found in the group treated with the high daily dose (125 mg/kg) – indicating a relationship between Co bioaccumulation and Fe concentration. The result is in agreement with [2,9] demonstrating negative correlation between Co accumulation and iron status. Chikh et al. also show that Co(III) compounds compete with Fe for the transferrin receptor [3]. Surprisingly, day 25 and day 30 mice had less iron in their blood compared to day 18 mice both in control and treated mice which is probably due to their growth and development.

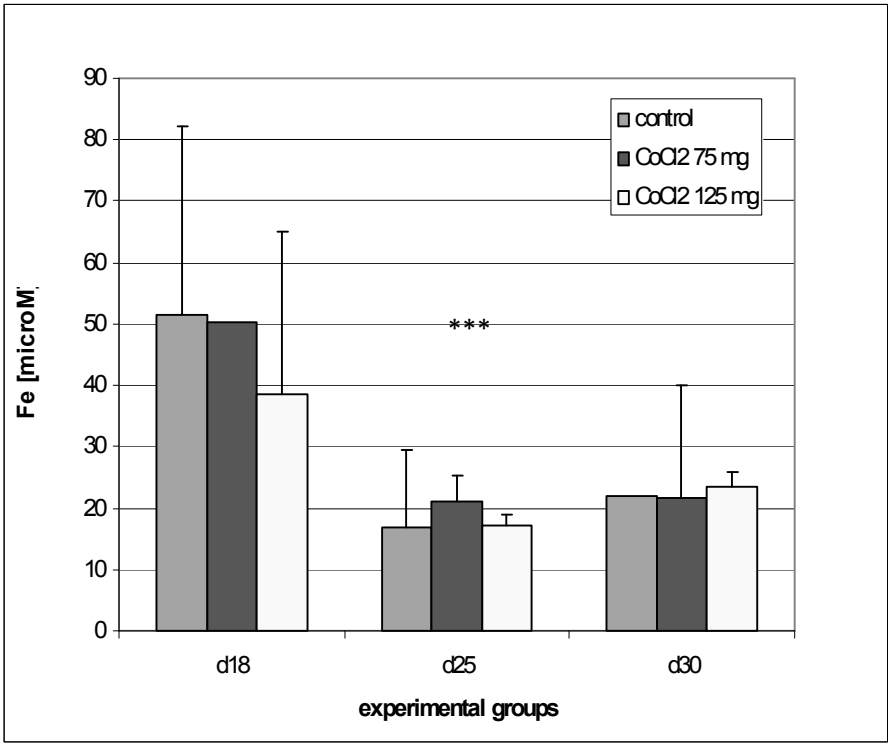

Fig.2. Fe concentration in blood serum. Each column represents mean±SD, n = 3. Triple asterisk (***) represents statistical difference ($p<0.001$).

A dose-dependent effect for Co(II) accumulation was also observed in the spleen (Fig.3). Day 18 mice were more sensitive to treatment having more metal ions in their spleen. The result could explain our findings for altered spleen index, extramedullar hematopoiesis in the spleen of Co-treated mice [7]. Day 25 and day 30 mice accumulate less Co(II) probably due to activation of specific enzyme systems. This indicates that the effect of Co(II) on young animals is very strong and long-term exposure can further cause serious organ damage and affect their development.

In the spleen of control mice more Fe was measured in day 18 animals compared to days 25 and 30 as observed in the serum samples. The same tendency was found in the metal-treated groups as well, and the accumulation of Co(II) in the spleen induced changes in the iron content. Elevated Fe concentration was measured in day 25 and day 30 of the Co-treated groups ($p<0.03$ for day 30 mice) compared to control samples. The increased iron storage in the spleen could explain the impaired erythropoiesis (reduced number of erythrocytes and hemoglobin content) observed in these groups (data not shown). Latunde-Dada et al. observed increased splenic Fe level in rats after phenylhydrazine treatment due enhanced erythrophagocytosis [11].

Liver is also shown to accumulate cobalt with a half-life of 10 years [15]. Chronic treatment with $CoCl_2$ induced significant increase of the metal ions in the liver (Fig.4). A time-dependent effect was observed having more Co(II) in the liver of day 30 mice, compared to day 18 and day 25 groups ($p<0.01$).

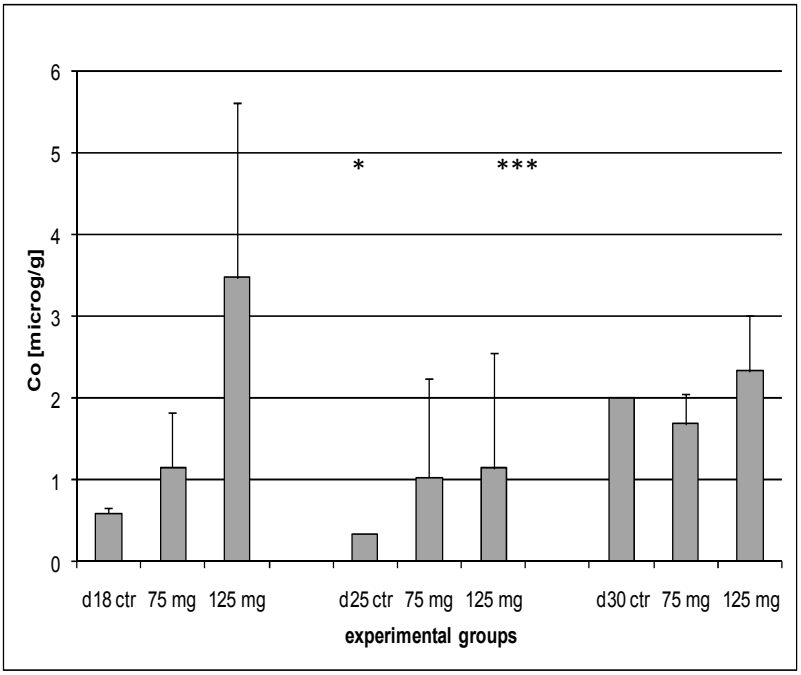

Fig.3. Co(II) concentration in the spleen of treated mice. Each column represents mean±SD, n = 3. Single asterisk (*) represents statistical difference ($p<0.05$) and triple asterisk (***) represents statistical difference ($p<0.001$).

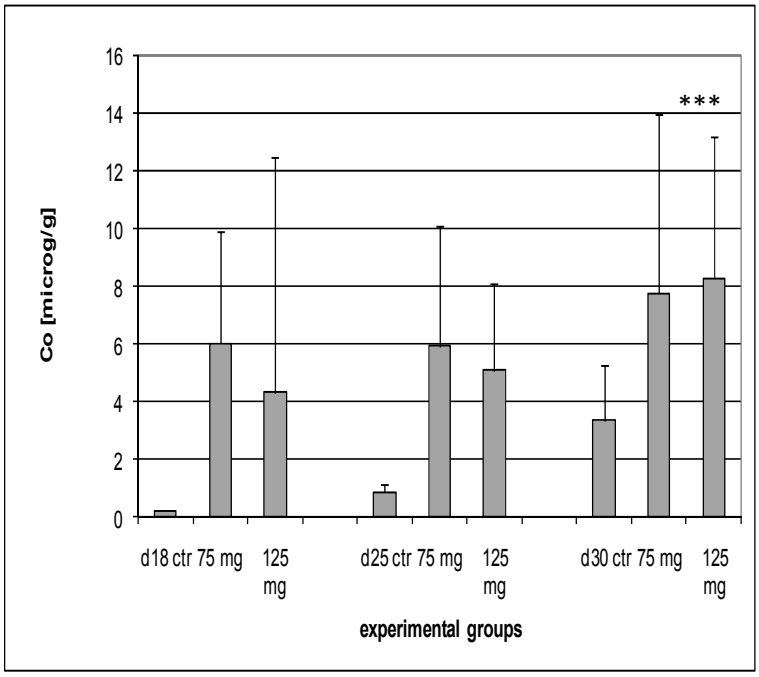

Fig.4. Co(II) concentration in the liver of treated mice. Each column represents mean±SD, n = 3. Triple asterisk (***) represents statistical differences ($p<0.001$).

As in the spleen, Co(II) accumulation resulted in increased iron storage in the liver as well. More Fe was measured in the Co-treated groups, compared to the controls. Increased iron content may lead to Fe overload which could induce free radical formation and generation of lipid peroxidation products resulting in tissue injury [12]. Results for altered Fe content confirm our previous data that chronic treatment with another cobalt compound - Co-EDTA induced significant changes in the liver and spleen of the exposed immature mice [8]. This indicates that the metal itself and not the ligand is responsible for the observed changes. Fe content in the liver is regulated by the protein hepcidin [12], therefore a possible affect of Co(II) treatment on hepcidin expression may be expected as well.

CONCLUSIONS

Exposure to $CoCl_2$ significantly increases Co(II) in blood serum, spleen and liver of treated immature mice compared to controls and induces changes in Fe content, thus affecting iron homeostasis. The elevated Co(II) content in the blood serum may be used as an early indicator for monitoring chronic exposure to this metal. The altered Fe content indicates a possible correlation between serum Co(II) and Fe concentrations having lower Co(II) at higher serum Fe content. Spleen and liver show different sensitivity to Co(II) administration. The observed changes indicate that immature mice are sensitive to Co exposure and could explain the hematological alterations in their red blood cell count, reduced hemoglobin content, increased values of hemoglobin-related parameters – MCH and MCHC. The three experimental groups of immature mice – day 18-, 25- and 30, used in the experimental design, show different sensitivity to the metal. This suggests that stage of development is also an important marker that should be considered.

Acknowledgements

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ВЛИЯНИЕ НА ТРЕТИРАНЕТО С КОБАЛТОВ ХЛОРИД ВЪРХУ МЕТАБОЛИЗМА НА ЖЕЛЯЗО ПРИ ПОЛОВО НЕЗРЕЛИ МИШКИ

Й. Глухчева, Е. Попова, В. Атанасов, И. Владов

Храната е основният източник на кобалт (Co) за населението. Въпреки токсичността му във високи дози, той е важен за бозайниците елемент в ниски концентрации. Третирането на животни с един химичен елемент обаче, често повлиява метаболизма и количествата на други елементи в тъканите. Целта на настоящото изследване е да установи влиянието на продължителното третиране на полово незрели мишки с кобалтов хлорид ($CoCl_2$) върху метаболизма на желязо (Fe). Бременни мишки са третирани дневно с дози 75 mg/kg телесно тегло или 125 mg/kg телесно тегло до 30-тия ден от раждането на мишлетата. На 18-, 25- и 30-ти ден новородените мишлета са използвани за анализ. Количествата на Co и Fe са измерени в кръвен серум, в слезката и черния дроб на третираните мишки. Първоначалните резултати показват, че въздействието с $CoCl_2$ повишава значимо количеството Co(II) в кръвния серум, слезката и черния дроб на третираните животни, в сравнение с нетретирани контролни мишки и предизвиква промени в концентрацията на Fe йони. Промените в количеството на Fe показват възможна връзка между серумните нива на Co(II) и концентрацията на Fe като при ниски стойности на Co(II) количеството на Fe в кръвния серум е по-високо. Слезката и черният дроб се повлияват в различна степен от третирането с $CoCl_2$. Промените показват, че полово незрелите мишки са чувствителни към действието на кобалта.

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