COBALT BIOACCUMULATION IN MOUSE BLOOD PLASMA AND LIVER

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ABSTRACT

Heavy metals such as cobalt are shown to accumulate in various organs of humans and animals. Oral exposure of immature mice to cobalt compounds (cobalt chloride and cobalt-EDTA) led to significant increase in cobalt (II) concentration in blood plasma and liver. 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 salts were dissolved and obtained from drinking tap water. Pure tap water was used as control. Mice were maintained in individual standard hard bottom polypropylene cages to ensure that all experimental animals obtained the required dose of cobalt salts. 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. Blood plasma and liver were used for measuring cobalt bioaccumulation. Cobalt (II) compounds showed differential bioaccumulation: higher concentrations were measured in the plasma compared to those measured in the liver. The effect depended on the type of compound used, dose, time duration as well as on the age of the experimental animals. Higher metal concentrations were detected in samples of mice treated with cobalt chloride compared to the samples exposed to Co-EDTA. The results indicate that day 18 mice are more sensitive to chronic exposure to cobalt compounds in high doses. Cobalt(II) concentrations in blood plasma may be used as a useful marker for diagnosing chronic exposure to cobalt compounds.

Keywords: cobalt compounds, bioaccumulation, mice, blood plasma, liver

Introduction

Heavy metals such as cobalt 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 (1).

Although cobalt is an essential trace element long-term exposure and large amounts of its salts can have deleterious effects on humans and animals. For the general population diet is the main source of cobalt. Cobalt(II) accumulates in organs such as kidney, liver, spleen, heart, stomach, intestines (2). Long-term exposure causes significant increase of cobalt levels in muscle, brain and testes as well. Exposure to cobalt causes allergic contact dermatitis, diseases of the upper respiratory tract, etc. (10). Absorption of cobalt in humans depends on the type and dose of the cobalt compound, the nutritional and iron status of the individual, etc. (12). Iron deficiency is shown to increase cobalt absorption in animal models as well as in humans. Young animals (rats and guinea pigs) have 3- to 15-fold greater absorption than adult animals (aged 200 days or more). In rats, cobalt chloride (with ^{58}Co tracer) that was complexed with histidine, lysine, glycylglycine, EDTA, casein, or glycine was absorbed less than free cobalt chloride. Cobalt chloride administered in conjunction with cow's milk resulted in significantly greater gastrointestinal absorption (~40%). Water-soluble cobalt compounds have been found to exhibit greater absorption than non-water-soluble forms (however, absorption of soluble cobalt compounds is higher and species dependent: in rats (13–34%), cows (1–2%), guinea-pigs (4-5%).

Co (II) compounds are shown to induce DNA damage,

DNA protein cross links, gene mutations, sister chromatid exchanges and aneuploidy in *in vitro* studies on animal and human cells (7).

In male Swiss mice, LD50 values ranged from 89.3 mg of cobalt (as cobalt chloride) per kilogram body weight to 123 mg of cobalt (as cobalt sulfate) per kilogram body weight (12).

As inorganic and complex compounds (with organic ligand) cobalt(II) is also used as nutritional supplement, preservative, in drinks, as therapeutic agent for treating different diseases, etc.

Ethylenediamine tetraacetic acid (EDTA) is a widespread organic pollutant. It is used in medicine, molecular biology and biochemistry; as anticoagulant for blood samples and decalcifying agent in histopathology, in non-alcoholic beverages. EDTA is a powerful antioxidant and due its ability to bind metals it is used in chelation therapy. 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 work is to study the accumulation of different cobalt compounds – cobalt chloride ($CoCl_2$) and Co-EDTA in blood plasma and liver of developing mice.

Materials and Methods

Complex synthesis

All chemicals and solvents used were of AR grade. Co-EDTA was synthesized according to modified literature procedures (5, 9) as already described (4).

In vivo 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 salts were dissolved and obtained from drinking tap water. 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 of cobalt salts.

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. Whole blood samples were obtained, centrifuged and plasma was stored at -20°C until further analysis. Liver was removed and used for measuring cobalt bioaccumulation.

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

Analysis of cobalt concentration in plasma and liver

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. Cobalt content in the liver was determined after nitric acid wet digestion and 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 at $p<0.05$ on SPSS software for Windows. Difference was considered significant at $p<0.05$.

Results and Discussion

Considering the fact that cobalt is transferred from food into the mother's milk we studied the effect of $CoCl_2$ and Co-EDTA on the newborn pups and its accumulation in blood plasma and liver.

Cobalt (II) concentrations in blood plasma

Chronic treatment with cobalt compounds ($CoCl_2$ and Co-EDTA) increased significantly Co(II) in blood plasma. After oral administration of $CoCl_2$ significantly increased Co(II) concentration was measured in blood samples of animals exposed to the high daily dose (125 mg/kg – **Fig. 1**). The same pattern was observed after treatment with Co-EDTA as well (**Fig. 2**). Significantly higher cobalt concentrations were measured in samples from animals treated with $CoCl_2$ compared to the complex Co-EDTA possibly due to the stability of the complex. The significance ranged from $p<0.03$ to $p<0.005$ for all experimental groups except day 18

mice treated with low dose cobalt compounds. The results are in agreement with data from WHO report showing that $CoCl_2$ is more absorbed than when complexed with EDTA. The results also confirm data from WHO (12) that young animals absorb more cobalt than the older ones. Treatment with high dose (125 mg/kg) $CoCl_2$ increased significantly Co(II) concentration in day 25 mice compared to those exposed to the low dose ($p<0.05$).

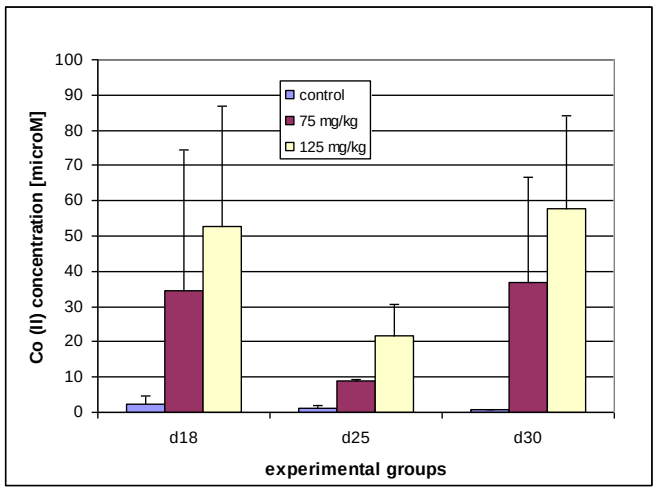

Fig. 1. Cobalt(II) concentration in blood plasma of mice treated orally with low (75 mg/kg) and high (125 mg/kg) daily doses of $CoCl_2$. Significant difference ($p<0.0001$) is found for day 25 low and high dose. Data represent as mean ± SD

Serum cobalt concentration mainly reflects recent exposure, as rapid renal excretion of cobalt is followed by a longer-lasting retention in tissues that is difficult to measure by noninvasive techniques (6).

Cobalt(II) concentrations in liver

Exposure of mice to cobalt compounds leads to accumulation of Co(II) in the liver. The highest metal ion concentrations were measured in the liver samples of day 18 mice. Significantly elevated concentrations of cobalt ions were measured in day 18 ($p<0.05$) and day 25 mice ($p<0.006$) treated daily with 75 mg/kg or 125 mg/kg $CoCl_2$. The high dose induced significant accumulation of Co(II) in day 30 mice ($p<0.02$) as well (**Figs. 3, 4**). These results could explain our and other researchers' data about reduced body and organ (liver) weight in animals exposed to $CoCl_2$ (3, 4, 11, 13). On the other hand, Liu et al. (8) demonstrate $CoCl_2$-hepatotoxicity in mice intraperitoneally injected with $CoCl_2$.

Liver samples of 18-day old mice treated with Co-EDTA showed significantly elevated Co(II) concentration ($p<0.02$) when high dose was applied compared to the low dose. Significantly elevated concentration of Co(II) was measured in day 25 for low ($p<0.004$) and high dose $p<0.04$) and for day 30 mice treated with low dose Co-EDTA ($p<0.02$).

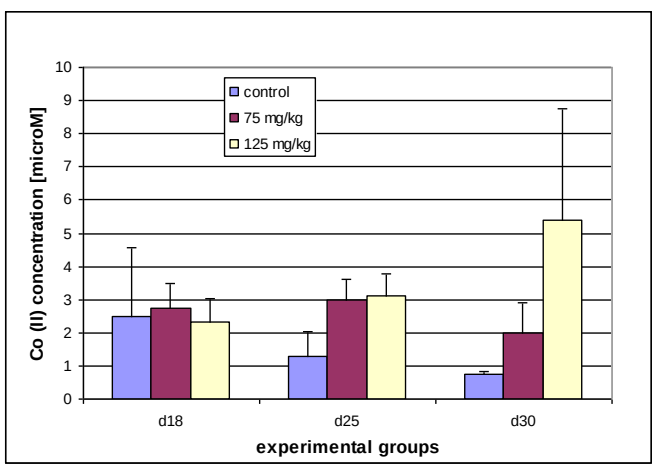

Fig. 2. Cobalt(II) concentration in blood plasma of mice treated orally with low (75 mg/kg) and high (125 mg/kg) daily doses of Co-EDTA. Significant difference ($p<0.02$) is found for day 25 mice for the two doses compared to the control samples. Data represent as mean ± SD

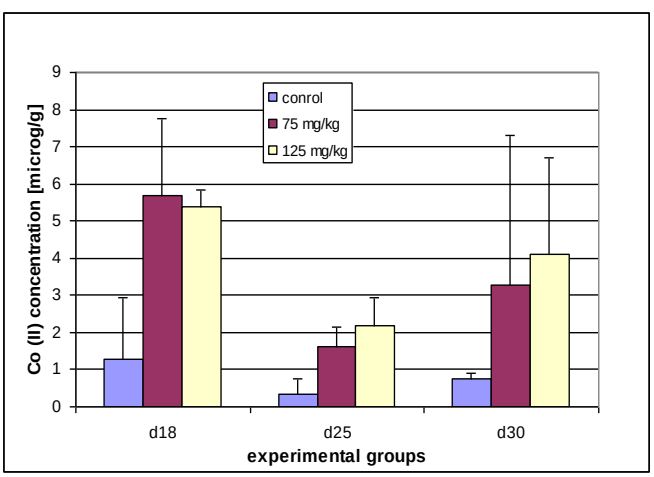

Fig. 3. Cobalt(II) concentration in liver of mice treated orally with low (75 mg/kg) and high (125 mg/kg) daily doses of $CoCl_2$. Data represent as mean ± SD

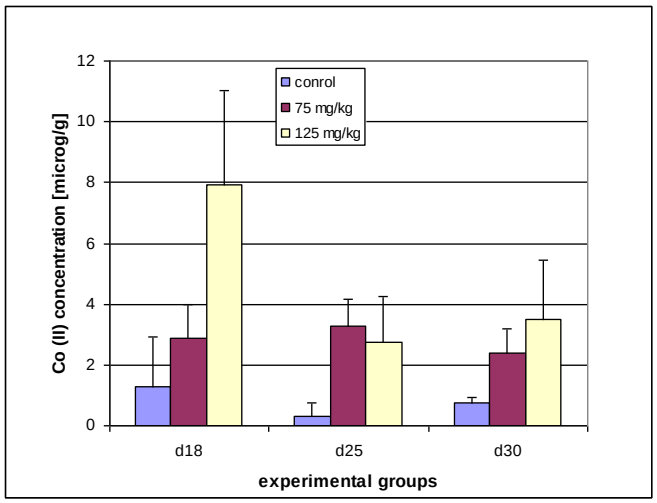

Fig. 4. Cobalt(II) concentration in liver of mice treated orally with low (75 mg/kg) and high (125 mg/kg) daily doses of Co-EDTA. Data represent as mean ± SD

Cobalt(II) concentrations were higher in livers of day 18 and day 30 mice treated with low dose $CoCl_2$ compared to those of animals treated with the same dose Co-EDTA.

Low dose Co-EDTA significantly increased ($p<0.004$) cobalt concentration in day 25 mice compared to the same dose $CoCl_2$.

The higher Co(II) concentrations in day 18 mice both in their plasma and liver could probably be due to their metabolism.

Conclusions

The biological activity of heavy metal compounds as well as their bioaccumulation is an important issue.

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. In general, day 18 pups accumulated more cobalt (II) in their blood plasma and livers compared to day 25 and day 30 mice. Cobalt(II) concentrations in blood plasma may be used as a useful marker for diagnosing chronic exposure to cobalt compounds.

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