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Effect of $CoCl_2$ treatment on major and trace elements metabolism and protein concentration in mice

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ABSTRACT

Cobalt (Co) is a transition metal and an essential trace element, required for vitamin B_{12} biosynthesis, enzyme activation and other biological processes, but toxic in high concentrations. There is lack of data for the effect of long-term Co(II) treatment on the concentrations of other trace elements. We estimate the influence of cobalt chloride ($CoCl_2$) on the relative content of different metals in mouse plasma using two-jet arc plasmatron atomic emission and on the total protein content. On average, the content of different elements in the plasma of 2-month-old *balb/c* mice (control group) decreased in the order: Ca > Mg > Si > Fe > Zn > Cu ≥ Al ≥ B. The treatment of mice for 60 days with $CoCl_2$ (daily dose 125 mg/kg) did not appreciably change the relative content of Ca, Cu, and Zn, while a 2.4-fold statistically significant decrease in the content of B and significant increase in the content of Mg (1.4-fold), Al and Fe (2.0-fold) and Si (3.2-fold) was found. A detectable amount of Mo was observed only for two control mice, while the plasma of 9 out of 16 mice of the treated group contained this metal. The administration of Co made its concentration detectable in the plasma of all mice of the treated group, but the relative content varied significantly. The treatment led to a 2.2-fold decrease in the concentration of the total plasma protein. Chronic exposure to $CoCl_2$ affects homeostasis as well as the concentrations and metabolism of other essential elements, probably due to competition of Co ions for similar binding sites within cells, altered signal transduction and protein biosynthesis. Long-term treatment also leads to significant weight changes and reduces the total protein concentration.

The data may be useful for an understanding of Co toxicity, its effect on the concentration of other metal ions and different physiological processes.

Introduction

Cobalt (Co) is an essential trace element required for vitamin B_{12} biosynthesis, other cobalamines, for enzyme activation. It is found in the environment, in food and water and therefore exposure to this metal is unavoidable. Although occupational exposure to cobalt occurs in several industries including hard metal manufacturing, welding, chemical industry, diet is the main source of cobalt for the general population [1]. Long-term exposure and large amounts of Co salts can have deleterious effects on humans and animals. Uptake and bioavailability of Co in humans depends on the type and dose of the cobalt compound, the nutritional and iron status of the individual, and some other factors [2]. Water-soluble Co compounds have been found to exhibit higher absorption than non-water-soluble forms, however, the absorption is species dependent [2]. Although toxic in high concentrations, Co is essential for mammals in low concentrations.

The biological effects of cobalt chloride ($CoCl_2$) are currently studied mainly with respect to its ability to modulate the activity of some antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, heme-oxygenase), to induce changes in the levels of lipid peroxidation and glutathione reduction during oxidative stress and to activate transcription factors [3,4]. Treatment with $CoCl_2$ leads to body weight reduction in normal and diabetic rats as well as to a decrease in plasma glucose levels in streptozotocin-diabetic rats [5,6].

Administration of one element to animals can often 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 [7]. Long-term exposure to $CoCl_2$ may possibly trigger a cascade of biochemical reactions,

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which will induce changes in signal transduction, protein biosynthesis and some 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 on one hand and affect animal development, on the other.

The aim of this study was to determine the effect of long-term exposure to $CoCl_2$ on the metabolism of some essential trace elements. Here, we have analysed simultaneously the effect of Co on the relative content of different metals (calcium – Ca, copper – Cu, zinc – Zn, magnesium – Mg, aluminium – Al, iron – Fe and molybdenum – Mo) and other chemical elements (boron – B and silicon – Si) as well as on the total protein content in the blood plasma of mice.

Materials and methods

Animals

Pregnant *balb/c* mice were subjected to daily dose of 125 mg/kg body weight cobalt chloride ($CoCl_2{\cdot}6H_2O$) 2–3 days before they gave birth to their mice. In our previous experiments this dose was shown to improve haematological parameters (will be published elsewhere). $CoCl_2{\cdot}6H_2O$ (0.35 mg/ml) was dissolved and obtained from drinking tap water. After birth, we continued to treat the mothers with the same dose because cobalt is transferred into the milk and thus the newborn mice were exposed to the metal ions. When the newborn mice ($n = 19$) were 25 days old they were separated into individual cages to ensure that all experimental animals obtained the required daily dose and treatment with 125 mg/kg body weight continued until they were 60 days old. The mice were weighed weekly and the Co concentration in the water was adjusted accordingly. In our previous experiments (will be published elsewhere) and experiments of this article no significant gender differences neither in body weight nor in haematological parameters were found and the experimental groups consisted of equal number of male and female mice. Haematological parameters for all mice (male and female) were obtained using automated haematological analyzer.

Animals were fed a standard diet and had access to the food *ad libitum* with strong control of the feeding regime. The mice were maintained in the Institute's animal breeding facility at $23 \pm 2\,^{\circ}C$ and 12:12 h light/dark cycle in individual standard hard-bottom polypropylene cages. The animals were sacrificed by decapitation after etherization. Whole blood samples were obtained, centrifuged, and the plasma was stored at $-20\,^{\circ}C$ until further analysis of protein concentration (see below). The blood sampling protocol confirmed to the local animal ethics committee guidelines. The control group consisted of 16 age-matched mice obtaining regular tap water.

Preparation of mouse blood plasma and metal content analysis

The two-jet plasmatron (10–15 kW) described in [8] was used. The analysis was carried out under the following experimental conditions: current strength – 80–85 A, plasma gas – 4 l/min, carrier gas – 0.7 l/min, angle between jets – 60°, analytical region – 4–5 mm lower than the point of the jet confluence. A diffraction spectrograph with a 600 lines/mm grating covering the spectral range of 200–410 nm was used. Spectrum registration was performed using a multielement photodiode analyzer of emission spectra produced by "VMK Optoelektronika" (Russia).

Calibration samples based on graphite powder containing 15 wt.% NaCl with the impurity concentration range of 0.01–100 μg/g were used to construct calibration curves. These samples were prepared from Russian State Certified Reference Material of graphite powder with different known composition of impurities (GSO 7751-2000, GSO 4519-89/4523-89; Ural State Technical University) by dilution of those with graphite powder containing 30 wt.% NaCl in the ratio of 1:1 in a clean room designed for manipulation with high-purity samples. The calibration samples are stable for at least a year. Standard errors (reproducibility) in the values are within 3–5%.

The mouse blood plasma was prepared by addition of 0.5 ml of 4% sodium citrate (Fluka) to 2 ml of fresh mouse blood. The mixture was kept for 24 h at 4 °C and the cells were removed by centrifugation. The plasma samples (1 ml) were frozen in liquid nitrogen, lyophilized and weighted. All powders (2–3 mg per analysis) were analysed for metal composition by two-jet arc plasmatron atomic emission according to [9]. Powders of lyophilized sodium citrate solutions (0.8%) were used as controls.

The mass percentage of metals and other chemical elements (% of the powder containing all dried components of plasma) was determined. The final mass percentage of each metal was estimated from the difference between the corresponding experimental and control powder samples. The data are presented as micrograms of chemical element per gram of every powder and then recalculated as mg of metal ions per 1 l of the plasma. Standard errors in the values are within 5–7%.

Protein concentration

The concentration of total protein in the blood plasma samples was measured using the Bradford assay [10].

Statistical analysis

The average values of all parameters analyzed (mean ± S.E.) were estimated using three independent assays for each sample of blood plasma. The differences between plasma samples of different groups were analyzed by Student's *t*-test, $p < 0.05$ was considered statistically significant.

Results

First we have determined the mass percentage of metals and several other chemical elements in the lyophilized plasma samples, calculated for 1 g of every lyophilized powder, using the two-jet arc plasmatron atomic emission method, which allows determination of many elements simultaneously. To our surprise, the masses of the lyophilised plasma samples from mice treated with $CoCl_2$ were on average 1.08-fold ($p < 0.05$) statistically significantly lower than those from the control group of animals. This factor was taken into account when estimating the relative content of different metal ions in the sera of treated and control mice calculated for 1 l of plasma (Table 1). Due to the small amount of powders, the content of some low-abundance metals (Ag, Co, Pb, Cr, and Ni) was below the sensitivity threshold of the method. The relative amount of different elements in the plasma of the control group of mice treated with Co^{2+} varied significantly between the individuals, the difference was in the range from 1.4- to 5.7-fold (Table 1). Interestingly, similar situation concerning a significant difference in the relative content of various metal ions was observed earlier in the case of the plasma from individual Wistar rats [11], and mouse organs [12].

One cannot exclude that a small decrease in the mass of lyophilised plasma samples from the treated mice is due not only by an increase in the content of some metal salts, but also by a change in the concentrations of other blood components with low or high molecular masses.

On average, the relative content of different elements in the plasma of the mouse control group decreased in the order: Ca > Mg > Si > Fe > Zn > Cu ≥ Al ≥ B (Table 1). The treatment of mice