Effect of Co-EDTA on hematological parameters in immature mice

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Abstract

As inorganic and complex compounds cobalt is used as nutritional supplement, preservative, in drinks, cosmetics, as therapeutic agent for treating different diseases. The aim of the present study is to investigate the effect of Co-EDTA on the hematological parameters of immature mice. Pregnant mice in late gestation were subjected to Co-EDTA treatment at daily doses of 75 mg/kg and the exposure continued until days 18 and 25 of the newborn pups. The compound was obtained from drinking water. Hematological parameters and serum iron (Fe) content were measured. Preliminary results show reduced red blood cell count (RBC), hemoglobin content (Hb), hematocrit (Hct) and mean cell volume (MCV) and increased MCH and MCHC in treated mice. Serum Fe was elevated in d18 mice and significantly reduced in d25 animals compared to age-matched controls. Fe content was in good correlation with RBC, Hb, Hct, MCV and showed strong inverse relation with MCH and MCHC in d18 mice which corresponds to the increased values of the latter parameters in the treated group.

Results indicate that treatment of immature mice with Co-EDTA induces significant hematological changes which probably alter their hemorheological properties as well.

Keywords: \textit{In vivo} mouse model, Co-EDTA, haematological parameters

1. Introduction

Cobalt (Co) is used as nutritional supplement, preservative, in drinks, cosmetics, as therapeutic agent for treating different diseases. Exposure to this metal also causes allergic contact dermatitis, diseases of the upper respiratory tract, etc. \cite{10}. Young animals (rats and guinea pigs) have 3- to 15-fold greater absorption than adult animals (aged 200 days or more). Water-soluble cobalt compounds exhibit greater absorption than non-water-soluble forms but absorption is species dependent \cite{15}. Although widely spread diet is the main source of cobalt (II) to humans and animals. The average daily intake of Co ranges from 5-45 μg with relatively high concentrations of the metal occurring in fish and in vegetables \cite{1}. 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 \cite{13}. Co is known to bind to the globin moiety of hemoglobin. Simonsen et al. \cite{12} show that human red blood cells (RBC) take up cobalt from the extracellular concentrations and the process is practically irreversible due to the highly effective binding to hemoglobin.

1.1. Cobalt-EDTA

Ethylenediamine tetraacetic acid (EDTA) is a widespread organic pollutant. It is a powerful antioxidant and due to its ability to bind metals EDTA is used in chelation therapy. In combination with sodium and calcium
EDTA has been used as the main therapeutic agent in case of lead poisoning for decades [2]. The compound finds wide use in medicine, molecular biology and biochemistry, beverages, in many ready-to-eat foods, cosmetics, etc. [6,8] As sodium iron EDTA it has been shown to increase iron bioavailability in human diets and has been proposed for use as a fortificant in certain grain-based products including breakfast cereals and cereal bars [4]. EDTA potentiates the mutagenic activity of chemical and physical agents in human and mice [6]. It is also cytotoxic and weakly genotoxic, but not carcinogenic. Oral exposures to EDTA produced adverse reproductive and developmental effects in animals [8].

Ruminal infusion of Co-EDTA alters milk fatty acid composition in lactating cows [11]. The wide use of cobalt compounds and EDTA requires full elucidation of their biological role on cells, tissues and organs after long-term exposure.

The aim of the study is to investigate the effect of Co-EDTA on the hematological parameters of immature mice.

2. Methods

2.1. Animal model

Pregnant ICR mice in late gestation were subjected to Co-EDTA treatment at daily dose of 75 mg/kg. The exposure continued until days 18 and 25 of the newborn pups. Co compound was dissolved and obtained from drinking tap water. Animals were fed a standard diet and had access to food ad libitum. Mice were maintained in individual standard hard bottom polypropylene cages to ensure that all animals obtained the required dose. They were maintained in the institute’s animal house at 23ºC ± 2ºC and 12:12 h light-dark cycle. Age-matched mice drinking pure tap water served as controls. The newborn pups were sacrificed on days 18 and 25.

Whole blood samples were obtained, centrifuged and serum was stored at -20ºC until further analysis. Hematological parameters — RBC, Hb, MCV, MCH and MCHC were measured on automated hematological analyzer BC-2800Vet (Mindray, China).

Serum iron (Fe) content was measured using “Iron Liquid” analytical kit based on Ferene-S as a chromogen (Sentinel Diagnostics, Italy).

2.2. Statistical analysis

The obtained results are presented as mean value ± SD. Significance between the experimental groups was determined using Student’s t-test at p<0.05. Correlation (r) between the iron content and the haematological parameters was studied.

3. Results

Cobalt (II) is transferred from the mothers to their offspring with the milk [7, 14]. Therefore the newborn pups are exposed to the metal during their nursing period and early development as well. Reduction in food or water consumption was not observed and all animals survived during the experiment.
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*** p<0.005

Chronic exposure of immature mice to Co-EDTA reduced significantly their RBC count compared to age-matched controls (Fig.1). This change was accompanied by a decrease in the mean corpuscular volume (MCV) of the erythrocytes (Fig.2). The latter alteration led to changes in the parameters dealing with the hemoglobin content – MCH and MCHC (Figs. 3, 4). Both indices were significantly increased in the treated group. The changes were more pronounced for day 18 mice indicating that they are more sensitive to treatment.

Fig.3. MCH in experimental mice. * p<0.05, *** p<0.005

Fig.4. MCHC in experimental mice. * p<0.05

Hemoglobin content (Hb) was reduced as well in the treated mice compared to the controls (Fig.5). The decreased RBC, MCV and Hb in Co-EDTA exposed mice are the basic signs of anemia. On the other hand, serum iron content was increased in day 18 mice and significantly reduced in the day 25 experimental group (Fig.6). A possible explanation is that some of the Fe was bound to EDTA, thus reducing its content in the serum of day 25 mice. The studies for the correlation between Fe and the haematological parameters revealed that treatment with Co-EDTA affects their relationships (Fig.7).
Differences in the correlations in the control groups indicate possible age-dependence. Fe content was in good correlation with RBC, Hb, Hct, MCV and showed strong inverse relation with MCH and MCHC in d18 mice which corresponds to the increased values of the latter parameters in the treated group.

4. Discussion

Cobalt is an essential amino acid required in the diet of the growing mouse [5]. As chloride it is used to treat anemia by enhancing endogenous erythropoietin production. Administration of CoCl₂ though, leads to body weight reduction in normal and diabetic rats as well as to a decrease in plasma glucose levels in streptozotocin-diabetic rats [16,17].

Treatment with EDTA has shown to induce severe biochemical and histopathological changes in bone marrow, liver, kidneys and testes of treated rats [6]. The compound led to a higher incidence of micronucleated polychromatic erythrocytes and chromosome aberrations in bone marrow cells in mice and Syrian hamster embryo cells [3,6]. The lowest dose reported to cause a toxic effect in animals was 750 mg/kg/day [8]. The dose
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 administration of Co-EDTA with drinking water led to reduced RBC, Hb, Hct and MCV in treated mice. MCH and MCHC were significantly increased in both groups (d18 and d25). These changes along with the reduced Hb content signify for disturbed erythropoiesis. This suggests that chronic and long-term treatment with the compound may induce anemia in young animals.

Serum Fe content was altered as well after treatment. It was elevated in d18 mice and significantly reduced in d25 animals compared to age-matched controls. Some of the free iron in day 25 mice could be bound to EDTA, due to the ability of EDTA to sequester metal ions. According to Khalil et al. it has become a valuable drug for the regulation of metal-ion concentrations in the biological systems, as well as for the removal of noxious substances from the human body [6]. Changes in the serum Fe concentration will affect not only Hb content as iron is a constituent of heme but other hematological parameters such as total-iron binding capacity, transferrin receptor expression, etc.

Co-EDTA-treated mice also showed different distribution of the iron in their spleen and liver (data not shown). Changes in the Fe content will affect not only erythropoiesis but oxidative metabolism and cellular immune response as well [9]. Co exposure also alters other elements’ metabolism which will further affect the growth and development of the treated group [18].

RBC and hematocrit are the main factors that affect blood viscosity. Our results indicate that treatment of immature mice with Co-EDTA induces significant hematological changes and therefore alterations in their hemorheological properties should be expected as well.

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References

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