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Changes in the mouse spleen after long-term treatment with cobalt(II) compounds

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Chronic treatment with cobalt(II) compounds induced changes in the spleen index of exposed mice. The compounds showed diverse effect on immature and mature animals. Administration of low and/or high doses of $CoCl_2$ or Co-EDTA reduced the index in d18 mice which proved that at this age they are the most sensitive.

Key words: cobalt(II) compounds, chronic exposure, *in vivo* model, mice, spleen

Introduction

The wide use of cobalt alloys in medical devices requires full elucidation of its biological role and cells, tissues and organs after long-term exposure [6, 9]. It accumulates in organs such as spleen, kidney, heart, liver, intestines, stomach, muscles, brain, testes, etc. [1]. Data show that cobalt is transferred from food into the mother's milk [7, 10]. Young animals (rats and guinea pigs) have 3- to 15-fold greater absorption than adult animals (aged 200 days or more) [11]. Although widely spread diet is the main source of cobalt(II) to humans and animals. The average daily intake of cobalt ranges from 5-45 μg with relatively high concentrations of the metal occurring in fish and in vegetables [2].

The spleen has a key role in hematopoiesis and in the immune response. In rodents the red pulp is a site for hematopoiesis especially in fetal and neonatal animals. Since it is also a storage site for iron, erythrocytes and platelets [3] alterations in its functions will affect iron metabolism, blood cell production, etc. The ratio of splenic weight to body weight remains fairly constant regardless of age [3].

The aim of the present study is to investigate the effect of long-term treatment with cobalt chloride ($CoCl_2$) and cobalt-EDTA (Co-EDTA) on the spleen of developing mice.

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

Material and Methods

Animal model

Pregnant ICR mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H_2O$) or Co-EDTA treatment at daily doses of 75 mg/kg or 125 mg/kg. Cobalt(II) compounds were dissolved and obtained from drinking tap water. Animals were fed a standard diet and had access to food *ad libitum*. Mice were maintained in the institute's animal house at 23°C ± 2°C 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 were sacrificed on days 18, 25, 30, 45, 60 and 90 which correspond to different stages of development. Mice were weighed weekly and the experimental cobalt concentration was adjusted accordingly. The spleens were excised, weighed and processed for histological analysis. Spleen index (SI) was calculated as a ratio of spleen weight to body weight.

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 $p<0.05$.

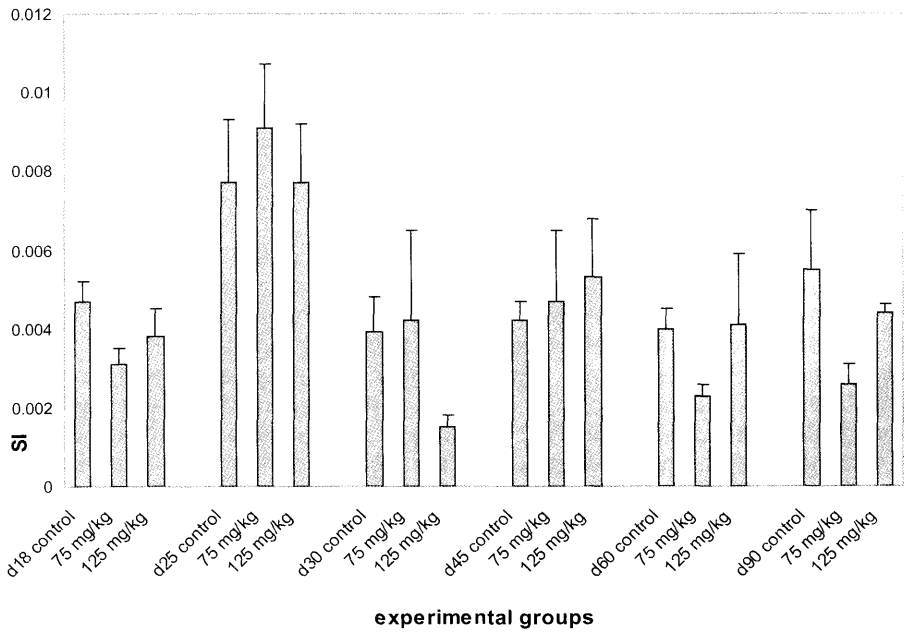

Fig. 1. Spleen index (SI) changes of mice treated with low and/or high daily dose $CoCl_2$. Each column represents data as mean ± SD. Asterisk (*) represents statistical difference $p<0.05$ and triple asterisk (***) $p<0.001$.

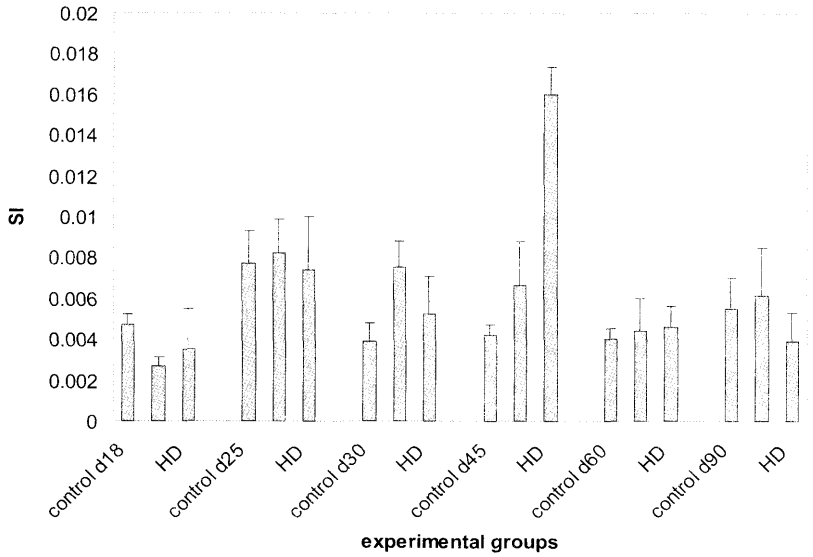

Fig. 2. Spleen index (SI) changes of mice treated with low and/or high daily dose Co-EDTA. Each column represents data as mean ± SD. Asterisk (*) represents statistical difference $p<0.05$ and triple asterisk (***) $p<0.001$. LD represents low dose (75 mg/kg) Co-EDTA and HD is for high dose (125 mg/kg) Co-EDTA.

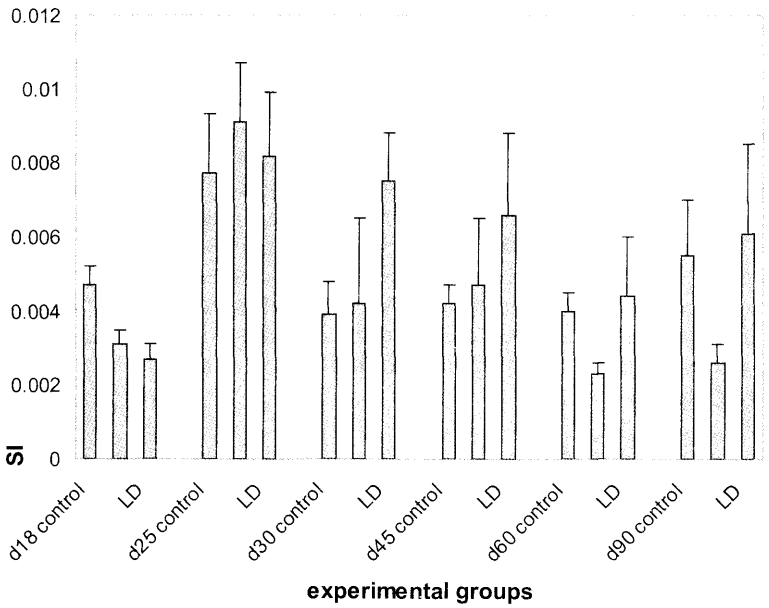

Fig. 3. Comparison of spleen index (SI) changes of mice after chronic treatment with low daily dose $CoCl_2$ and/or Co-EDTA. Each column represents data as mean ± SD. Asterisk (*) represents statistical difference $p<0.05$. LD represents low dose (75 mg/kg) Co-EDTA.

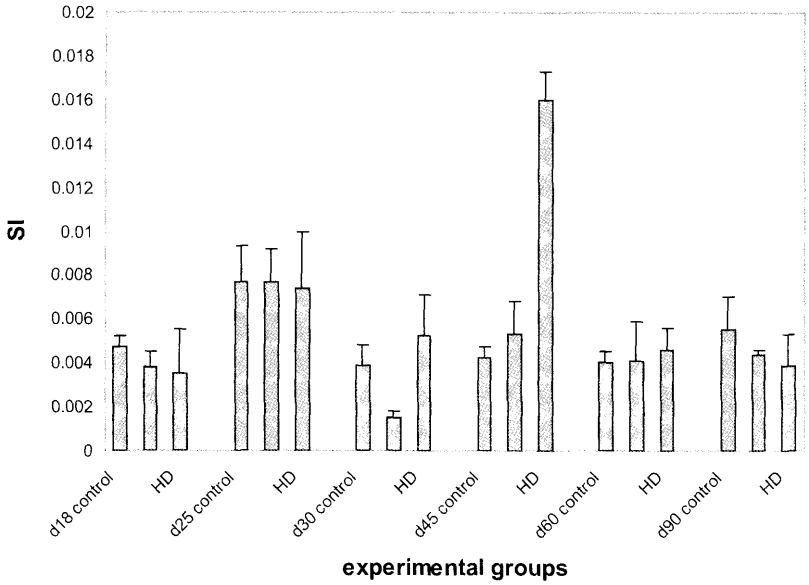

Fig. 4. Comparison of spleen index (SI) changes of mice after chronic treatment with high daily doses $CoCl_2$ and/or Co-EDTA. Each column represents data as mean ± SD. Triple asterisk (***) represents statistical difference $p<0.001$. HD stands for high dose (125 mg/kg) Co-EDTA.

Results and Discussion

Long-term exposure to Co(II) compounds induced changes in the spleen index (Figs. 1,2). SI was significantly decreased in immature mice. Day18 and d25 treated with $CoCl_2$ have increased SI compared to Co-EDTA (Figs.3,4). In mature mice (d30 – d90) treatment with Co-EDTA increased SI. Our results are in agreement with Simonyte et al. [8] and Dkhil [4] demonstrating increased SI in mice after long-term exposure to heavy metals and infections. Increased SI is associated with changes both in the white and red pulp [4]. The increased splenic index in our studies could be due to increased number of macrophages in the spleen. As a transition metal cobalt enhances oxidative stress which can further damage the organ. The results could explain the observed by us disturbed extramedullar hematopoiesis in the spleen after long-term treatment of mice with low or high dose Co-EDTA [5].

Conclusions

Long-term exposure to $CoCl_2$ and Co-EDTA alters SI of treated mice. The compounds affect differently immature and mature mice. They reduced SI in d18 mice which are the most sensitive.

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