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In vivo Effect of Heavy Metals Cd, Pb, Cu and Zn on Mice Spermatogenic Cells and Chromosome Reactivity

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ICR mice were given a diet enriched of heavy metals Cd, Pb, Cu and Zn for 15, 40, 60 and 90 days. The ultrastructural alterations in male germ cells and chromosome reactivity were studied. Spermatogonia and spermatocytes were found the most sensitive cells. The disturbances were seen predominantly in the cytoplasm and nuclei of the spermatocytes with dilatation of the endoplasmic reticulum cisternae and chromatin condensation. The tripartite structure of synaptonemal complexes have shown significant persistence. Chromosome aberrations of chromatide and isochromatide type, Robertsonian translocations and changes in chromosome spiralization were significantly increased in all experimental groups. There was no direct correlation between duration of the treatment and the percentage of aberrant mitoses. The presence of metaphases with altered coiling and "C-banding" effect related with changes in chromatin spiralization were confirmed by ultrastructural observation on spermatogenic cells.

Key words: heavy metals, spermatogenesis, chromosomes.

Introduction

The drastic increase in number of toxic chemicals in environment results irreversible adverse effects in animals and human beings. Reproductive system is very sensitive to different environmental factors and could be used as a useful tocl to solve many ecological problems. Due to complexity and long duration of the spermatogenic process, the testes of mammals are highly sensitive to damage produced by environmental expose to chemicals [5]. The polymetallic industrial dust as a waste product in lead-zinc factory near Assenovgrad (Bulgaria) is very rich in heavy metals Cd, Cu, Pb and Zn. In our previous article we have published the bioaccumulation of the above heavy metals in liver, kidney, spleen and bones in mice [6] and the effect on body weight, blood, oxygen consumption, mitotic index etc. [7]. In the present study we focus on the in vivo effect of Cd, Cu, Pb and Zn on subcellular structure of spermatogenic cells and chromosome reactivity in mice under ecologo-toxicological experiment.

Material and Methods

Male and female 10 weeks old ICR mice were given a diet summarized in Table 1. Polymetallic industrial dust from electrofilters of the lead-zinc rafinery near Assenovgrad (Bulgaria) was mixed mechanically at 1% ratio from conventional animal food. Samples were taken at 15, 40, 60 and 90 day of treatment. Two hours before sacrification mice were given C-methyl 3H-thymidine (NEW Products, Boston, MA)(spec act. 20mCi/mmol) in a dose of 2 μCi/g of body weight. Pieces of testes were proceeded for routine histological, autoradiographic and ultrastuctural study. Chromosome preparations "slides" were obtained from colchicine blocked bone marrow cells (2-4 g/kg b.w.) according to a routine protocol [4]. Air dried slides were stained with 5% Giemsa solution.

Table 1. Quantity of heavy metals (mg/kg) in the diet of mice

Group	n	Cadmium	Lead	Cooper	Zinc
Experimental	5	64.1± 10.5	784± 244	20.9 ±7.3	1945±429
Control	5	3.5 ±1.9	61.8± 20.9	1.3 ±1.1	90.9± 25.9

Results and Discussion

Histological assessment did not show significant differences between the testes of control and experimental animals. On the autoradiographs the percentage of labelled spermatogonia in control mice was 42±3 without significant difference between control and experimental groups with exception of day 40—20±2% labelled spermatogonia.

At the ultrastructural level in the experimental groups different alterations were visible. Spermatogonia and spermatocytes were the most affected germ cells and could be seen in different degree of destruction. In some of them the cytoplasm was highly vacuolized and the chromatin was condensed in amorphous heterochromatin accumulations. Fully destructed spermatocytes were obvious. The tripartite structure of synaptonemal complexes (SC)was well preserved even in completely degenerated pachytene spermatocytes. The higher resistence of SC was observed after in vivo x-ray irradiation due to a high degree of chromatin condensation and the strength of DNA-protein complexes [3] The cytoplasm of some Sertoli cells was rich in lysosomes as an expression of increased phagocytic activity.The higher bioaccumulation of heavy metals in liver, kidney and bones at 40, 60 and 90 day [6] coincides with the above described ultrastructural alterations of spermatogenic cells. Obviously the excess exposure of the organism to heavy metals disturbs the whole homeostasis including reproductive system. More studies are required to follow the effect of interactions between several heavy metals like Cd/Zn, Cd/Cu, Pb/Cu on the process of spermatogenesis.

Analysis of the metaphases preparations including structural and numerical changes was carried out both on control and treated mice. Chromosome aberrations were of chromatide (breaks and fragments) or isochromatide type — the first prevailing. The presence of ring chromosomes and Robertsonian translocations was also noticed. The analysis of characteristics of the chromosome aberrations suggests they are the results of clastogenic effect of Pb++ as a basic component of applied industrial dust [1, 2]. Metaphase analysis has shown a lack of direct correlation

between duration of the treatment and the total percentage of aberrant metaphases. The constancy in harvested aberrations in rapidly proliferating bone marrow cells could be explained with an equilibrium between the process of induction of observed aberration by the mutagens and the elimination of the aberrant cells in the course of mitotic division.

More than 5% of analysed metaphases in the treated animals have shown changes in the spiralization of chromosomes and clear "c-banding" effects most probably related with changes in the chromatin spiralization. The highest percentage of metaphases with altered spiralization and "C-banding" effects at 15th day of treatment indicates an adverse influence of the heavy metals on chromatin structure, confirmed by ultrastructural observations on spermatogenic cells.

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