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Effect of lifetime low dose exposure to heavy metals on selected serum proteins of Wistar rats during three subsequent generations

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ABSTRACT

The aim of the study was to assess the effects of exposure to low doses of lead, cadmium and mercury dissolved in drinking water (at a concentration 200-fold of maximum allowable concentration) on selected serum proteins of 120 Wistar rats during three subsequent generations. Animals were divided into four groups in all observed generation—control (C) and three experimental groups exposed to low doses of heavy metals (lead acetate in concentration 100 μM; mercuric chloride in 1 μM; cadmium chloride in 20 μM of drinking water). We studied the biochemical parameters as well as total protein, albumin, transferrin and ferritin in the serum. Exposure to lead and mercury shortened life span, decreased body weight of the animals in each generation whereas cadmium had no such effect. Total protein increased after exposure to lead and mercury ($P<0.001$), albumin increased after exposure to lead and mercury in 1st filial and 2nd filial generation ($P<0.05$) and insignificantly decreased after exposure to cadmium in parental and 1st filial generation. Transferrin and ferritin increased in all exposed groups and generations ($P<0.05$). Transferrin and ferritin are good markers for intoxication of rats with heavy metals. For the results evaluation, not only data at the end of experiment should be taken into account, but entire duration of trials (i.e., more time steps), which makes results more objective.

1. Introduction

Constantly increasing environmental contamination is a dangerous accompanying feature of industrialized world but also of developing countries. Important and nondegradable environmental contaminants are heavy metals (mainly lead, cadmium and mercury). Therefore, the contamination of the environment, including drinking water sources is still a very topical problem (Massányi et al., 2004; Cigánková et al., 2010).

It is a lot of controversy about the exact definition of which elements belong to the heavy metals. Some definitions tend to define them according to atomic weight, or by specific gravity (density from 4.0 to 5.0 and above). According another view the term "heavy metals" refers to those metals and metalloids, which pose an environmental risk to living creatures (precisely they are toxic for humans and for animals). One also should take into account that only some of these elements are abundant in soil and water.

Monitoring the exposure to these elements and revealing the first signs of subclinical intoxication is important not only from medical point of view but has also important implications for the health policy measurements. Reliable and generally useful markers for early detection of exposure to heavy metals are not developed yet. Therefore the exposure often remains undetected and can lead to unforeseen and unexplained increased morbidity and mortality in some regions of the world (Liu et al., 2009).

Toxicity of heavy metals depends on many factors (Lukáč et al., 2009). Specific symptomatology depends mainly on the characteristics of the element determining its effect on biological systems. Some elements vary significantly in their toxicity depending on their chemical form. Among other factors the total absorbed dose and the time course of exposure (acute or chronic) are of great importance. Toxicity is also influenced by age, general health status and nutrition of the affected persons. For example small children are more sensitive to lead exposure (Rosin, 2009), because they absorb several times more from a given amount and their brain is more sensitive to exposure (Henretig, 2006). Particularly important is the gate of entry into the body. Mercury in the elemental form is relatively inert in the gastrointestinal tract and is poorly absorbed through the intact skin. On the other side inhaled mercury vapors can cause disastrous consequences.

Almost all organ systems are involved in heavy metal toxicity, although the most common are central nervous system (Bowler et al., 2007), peripheral nervous system, gastrointestinal tract,

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haematopoietic system, kidneys and cardiovascular system (Prozialeck et al., 2008; Ozturk et al., 2009).

Heavy metal exposure usually occurs through food chain, drugs, environment or occupational microenvironment and all these factors influence its outcome. Knowledge of macro- and microenvironmetal situation, dietary habits and lifestyle together with the regular monitoring of the effects can be the basis of preventive measures in this particular field.

The main objective of this study was to investigate basic physiological data (body weight, food and water intake as well as life span) and selected serum proteins in relation to lifetime exposure to Wistar rats with low doses of lead, cadmium and mercury during three subsequent generations.

2. Material and methods

Experiments were conducted in two arms: reproductive trial and principal trial. In this work presents results only from principal trial.

2.1. Animals, conditions and experimental protocol of principal trial

120 Wistar rats (male, 52 days old, average weight 166 ± 27 g) were obtained from the SPF breed of CAL FM UPJŠ for P (parental) generation ($n=40$) as originated for F1 (1st filial generation, $n=40$ as progeny from P generation of reproductive trial) and F2 (2nd filial generation, $n=40$ as progeny from F1 generation of reproductive trial) from the same treated group. Rats were included into 4 groups ($n=10$ males) in each generation. The control group (C, $n=10$) received pure drinking water. The second group (Pb, $n=10$) received lead acetate in concentration of 100 μM in drinking water. The third group (Cd, $n=10$) received cadmium chloride dihydrate in concentration of 20 μM in drinking water. The fourth group (Hg, $n=10$) received mercuric chloride in a concentration of 1 μM. This arrangement was applied also to the F1 and F2 generations. Rats during the experiment were kept individually in whole-glass metabolic cages with free access to tap water and food from age 52 days (0th day in principal experiment). Arrangements of the experiment are shown in Fig. 1.

Experiments were terminated on the 156th week of experiment in each examined generation. The animal room was designed to maintain temperature at 22 ± 2 °C, relative humidity at approximately 50%, and a 12 h light:12 h dark photoperiod. Experiments were conducted in the CAL FM UPJŠ, Košice, Slovak Republic, which is accredited to the breeding of laboratory animals and for animal experiments in accordance with the relevant legislative provisions. Experiment was approved by Ethical Committee of Faculty of Medicine and State Veterinary and Food Administration of Slovak Republic (rec. Ro-7879/04-220/3).

Every 26th week of experiment, we estimated survival, mean life expectancy, body mass, food intake, water intake and levels of total protein, albumin, ferritin and transferrin in serum. Blood sampling was done every time at morning between 07^{00} and 09^{00}.

2.2. Experimental protocol of reproductive experiment

80 Wistar rats (40 males and 40 females, 4 weeks old, average weight 120 ± 19 g) were obtained from the SPF breed at the University of Šafárik, Faculty of Medicine, Central Animal Laboratory (CAL FM UPJŠ) and used as parental generation (P) in a reproductive experiment (the results of this experiment in this work not present) or individuals for F1 and F2 groups of presented experiments, and were also divided randomly into four groups, control and three exposed groups (10 males and 10 females in each group). The groups were treated identically as in the principal trial. This arrangement was applied also to the F1 generation (progeny from same group of P generation) and F2 generation (progeny from same group of F1 generation).

The rats in reproductive trial were kept in metal-free cages (1 female and 1 male per cage). All groups were fed with the same standard food. Reproductive trial was terminated on the 78th week of trial in all generations.

2.3. Biochemical analysis

Sample preparation: Blood was collected from the tail vein. Samples of blood (500 μL) were allowed to clot at room temperature for 1 h, centrifuged at 1000*g* for 45 min at 4 °C and the sera stored at −24 °C. Sera were not used if hemolysis was present. We investigated the following parameters:

Total proteins. We used a biuret method for its simplicity, speed and reliability, with modification by Flack and Woollen (1984).

Albumin, transferring, and ferritin determination was performed by commercial test from Dot Diagnostics Ltd., Czech Republic.

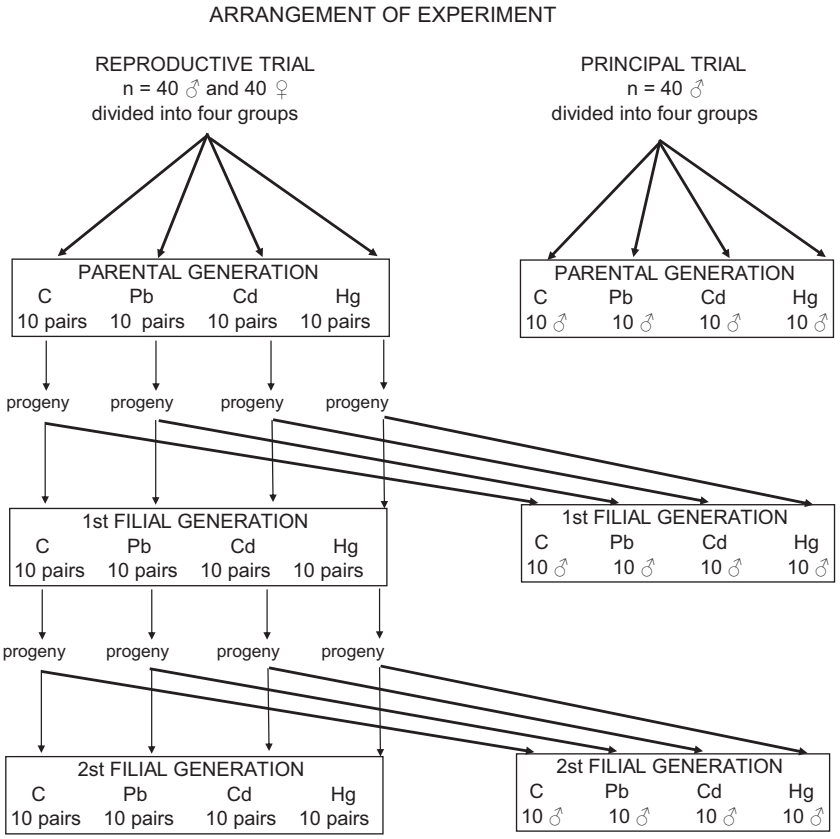

Fig. 1. Arrangement of experiment and distribution of rats into groups.