HISTOARHITECTONICS OF OVARY AND UTERUS AFTER LONG-TERM EXPOSURE TO LOW LEAD LEVELS IN FEMALE RATS

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Introduction

Lead is a heavy metal widely dispersed in the environment, which remains in biota for long time periods. High levels of exposure can occur especially in industrial areas, more present in the developing countries. In contrast to other metals, lead does not have any physiological role in the body and there isn’t a known minimum level that would be considered as non-toxic. Lead can affect all great systems from the cardiovascular, gastrointestinal, urinary and nervous to reproductive systems. Usually exposure to lead is via dermal, oral, or by inhalation, lead being also involved in the transplacental congenital intoxication, with the following consequences. Generally, the mammalian females’ reproductive toxicology information available is sparser than those for male, because of differences in gametes genesis and of cyclic nature of female’s reproduction function.

Aim of present study appeared following our anterior researches in rat females, in this topic: three months study (lead levels in organs and tissues, hormone levels and sexual cycle duration); in utero exposure; suckling and pre-pubertal exposure and respectively two generation study in female rats, to continue to follow lead’s accumulation levels in the ovaries, Fallopian tubes and uterus in a 12 months chronic exposure study 1-6.

Materials and Methods

Animals

Animals were purchased from the authorized biobase of the “Victor Babes” University of Medicine and Pharmacy from Timisoara. It was in our concern to choose, as possible, all females with the same body weight, the average weight for experimental groups being E1 = 220.0 ±5.7 g; E2 = 225.5 ±1.16 g; E3 = 220.0 ±5.80 g, and respectively C = 221.00±0.83 g. Before starting the experiment, rat females were subjected to a seven-day period of acclimatization and their health was confirmed clinically. The animals were kept in the same experimental room for all experimental period. Rat females were housed in four polycarbonate cages with 750 x 720 x 360 mm (L x w x h) dimensions. Eight females each cage / group. As bedding wood shaving was used. The environmental temperature was maintained at 20 ± 2 °C and relative humidity of 50 ± 10%. During experimentation period, the light cycle was 12 hours light and 12 hours dark. Non-sterile pelleted diet (Diet, Biovetimix, code 140-501, Romania) and tap water were offered ad libitum.

The evaluation of reproductive system integrity of leads toxic effect biomarkers was carried out on 20 white Wistar female rats of sexual maturity (120 life days), divided in four groups: three experimental (E1, E2, E3) and one control (C). The individuals from E groups were exposed to lead, as soluble lead acetate (Merck, Germany) consumption, in drinking water, twelve months, after the exposure to 0.050 mg L -1 lead, areas with optical empty spaces were present in the ovary tissue followed by diffuse oedemas and ovarian follicles denudation (figure 4).

Organs collection and histological examination

Ovaries, Fallopian tubes and uterus were freshly collected and fixed in alcohol (80 vol.) to be prepared histologically. After sampling, histological examination was accomplished. Fragments of tissue were fixed in alcohol 80%. Paraffin blocks containing tissue fragments were sectioned on microtome, resulting in 5 μm thick sections. Sections were stained by hematoxylin & eosin method using the hematoxylin-eosin (H&E) method. Histological images were captured by using Olympus CX 41 software program, at the magnification of X 40-100.

Samples digestion and Atomic Absorption Spectrometry

Digestion was accomplished using CEM Mars X micro wave digestion oven (CEM Microwave Technology Ltd. UK). Samples of sexual organs of 1.0 g were put into digestion flasks with 10 ml nitric acid (Merck, Germany) and 5 ml of perhydrol, at 600 W, for 20 minutes at 120°C. Evaluation of lead’s levels in sexual organs was accomplished after digestion by Atomic Absorption spectrometry (AAS), using an AAS 400 Zeeman, with graphite furnace (Varian instruments Inc. USA), programmable sample d. (PSSD 120) (with standard limit of 0.06 μl). Absorbance was determined by peak measuring and the concentration was determined based on New Rational calibration algorithm, in μg units. Calibration curve was made to a 283.3 nm wavelength, on five standard lead element levels, in HNO3 0.1%, with Stilt Width of 9.5 nm. To every 10 determinations, recalibration was made, (RSD < 10%).

Results

Results of lead levels found in rat female organs: ovary, uterus and Fallopian tubes, after 12 months chronic exposure to the lead acetate are presented in table 1.

Table 1. Lead concentration of (μg/g) in tissues and organs / groups

<table>
<thead>
<tr>
<th>Organ</th>
<th>Lead levels (μg/g)</th>
<th>Confidance intervals (mean ± SD)</th>
<th>283.3 nm</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>111.40±3.77***</td>
<td>10.68</td>
<td>120.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83.29±2.79***</td>
<td>7.90</td>
<td>76.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.18±0.76ns</td>
<td>2.15</td>
<td>13.38</td>
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<td></td>
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</tr>
<tr>
<td>uterus</td>
<td>111.40±3.77***</td>
<td>10.68</td>
<td>120.3</td>
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</table>

Fig. 1 Lead levels in ovary and in uterus and Fallopian tube.

In Figure 5, there can be seen necrosis zones in uterus consequently to 0.050 mg L -1 lead exposures. After the exposure to 0.100 mg L -1 lead the rat females’ ovary presented large zones of necrosis and follicular edema (figure 6).

Conclusions

The results of our research on chronic exposure of the rat females to lead acetate in drinking water identified significant increase of lead levels in the ovaries, Fallopian tubes and uterus, comparatively to the control group linked to the exposure level and followed by the modification of reproductive system integrity biomarkers.

Main structural changes found in ovary were: diffuse edema, necrosis in the ovarian follicles and different stages of evolution (A), with PF (primordial follicles) and A/PH (animal follicles). H&E stain, X100.

Fig. 2 Histological investigation of rat’s ovary from Control group revealed the presence of ovarian follicles in different stages of evolution (A), with PF (primordial follicles) and A/PH (animal follicles). H&E stain, X100.

The histologic sections made on rat females’ ovary after exposure to 0.150 mg L -1 lead revealed the heaviest changes of this organs, especially oedemas and necrosis of the ovarian follicles (figure 8). Finally, the microscopic examination of the uterus after exposure to respectively 150 ppb Pb revealed also the necrosis of the uterine glands (figure 9).

Fig. 7 Histological section in rat’s ovary after exposure to 0.100 mg L -1 lead; H&E stain X 100 (NCS- necrosis of uterine glands).

References: 34 titles