Selenium protects reproductive system and foetus development in a rat model of gestational lead exposure

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Abstract. – OBJECTIVE: Lead is a common environmental contaminant in the body especially dangerous for pregnant women and newborns. Selenium is a trace element which may rectify the damaging effects of lead. Here we tested potential protective effects of selenium against gestational lead exposure.

MATERIALS AND METHODS: Pregnant SD rats were exposed to 200 mg/L of lead acetate (given with water), with or without sodium selenite supplementation (2-8 mg/kg/day via intragastric administration). Pregnant rats not exposed to lead or selenium served as control animals. The outcomes in pregnant rats were serum lead and selenium levels, reproductive hormone (follicle-stimulating hormone, luteinizing hormone, prolactin, oestradiol, progesterone) levels, and uterine and ovarian morphological changes. The outcomes in the offspring were sex differentiation, survival rates (day 21 after birth), weight (days 0-35 after birth), weight of reproductive organs, and puberty onset (foreskin separation or vaginal opening).

RESULTS: Selenium supplementation dose-dependently decreased serum lead levels, rectified reproductive hormone levels, and attenuated reproductive morphological changes caused by lead exposure. Lead exposure did not affect sex differentiation, but significantly (p < 0.05 vs. control animals) decreased the offspring weight on day 0-28 and the weight of their reproductive organs. Furthermore, lead exposure delayed the onset of puberty. These pathological changes were dose-dependently rectified or attenuated by selenium supplementation.

CONCLUSIONS: Gestational lead exposure causes damage to the reproductive system of pregnant rats, and negatively modulates growth and reproductive system development of the offspring. These adverse effects are rectified or attenuated by selenium supplementation.

Key words: SD rats, Lead, Selenium, Reproductive hormones, Reproductive system, Puberty onset.

Introduction

Lead is a common environmental contaminant toxic to humans. Lead accumulation in the body causes systemic damage, especially in pregnant women and their newborns. Excessive serum lead levels are associated with increased risk of premature membrane rupture, prematurity, gestational hypertension, spontaneous abortion, and disturbances of foetal growth and development. Therefore, it is important to prevent the damaging effects of lead on reproductive system of pregnant women and on foetal health.

Selenium is a trace element essential for human and animal health. Selenium is present ubiquitously in the body and is involved in metabolic processes. During pregnancy, selenium levels gradually decrease, reaching the minimum just before the delivery. It is plausible to hypothesize that adverse effects of lead during pregnancy may be related to selenium deficiency. Conversely, selenium supplementation may reverse the damaging effects of lead. In this study, we exposed pregnant SD rats to lead with or without selenium supplementation. The outcomes were changes of reproductive system of pregnant female rats, and body growth and reproductive system development of their offspring. We demonstrate below beneficial effects of selenium supplementation on these parameters.

Materials and Methods

Reagents and assays

Sodium selenite (Na₂SeO₃) was purchased from Beijing United Chemical Co. (Beijing, China). Lead acetate ([Pb(C₂H₃O₂)]₄) was obtained from Guangzhou Chemical Reagent Factory.

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As downstream assays, we utilized five magnetic microparticle radioimmunoassay assays (iodine [125I]-human follicle stimulating hormone, 125I-human luteinizing hormone, prolactin, 125I-oestradiol, and 125I-human serum progesterone), purchased from Beijing Beimian-Dongya Institute of Biological Technology (Beijing, China).

**Instruments**

H-7500 transmission electron microscope was obtained from Hitachi (Tokyo, Japan), and WFX-120 atom spectrophotometer and AF-610A atomic fluorescence spectrometer were obtained from Beijing Ruili Analytical Instrument Company (Beijing, China). HMIAS-2000 high-resolution color medical image analysis system was obtained from Wuhan Qianping Imaging Technology Company (Wuhan, China), whereas SN-684 type γ RIA counter was from the Shanghai Institute of Nuclear Research (Shanghai, China).

**Animals**

Animal experiments conducted in this study were approved by the Animal Research Committee of Hebei Medical University and were conducted in accordance with the guidelines of the Animal Experimental Center of Hebei Medical University.

One hundred and eighty adult SD rats (60 males and 120 females, weight 240-270 g) were used in this study. The animals were purchased from the Experimental Animal Center of Hebei Medical University (Certificate of Conformity: DK0503-0031). The animals were incubated at ambient temperature of 20 ± 2º C, humidity of 50 ± 20%, and a circadian cycle of 12 dark/light hours. Male and female SD rats were placed in the same cage everyday at 20:00. At 8:00 each morning, vaginal plugs of female rats were assessed, and vaginal smears were procured. Smears positive for sperm were used as an indication of pregnancy.

**Exposure of pregnant rats to lead and selenium**

One hundred pregnant SD rats were randomly divided into five groups (20 rats/group). The groups were control group, lead-exposed group, lead + low dose of selenium group, lead + intermediate dose of selenium group, and lead + high dose of selenium group. Beginning from the first day after conception, the rats in the lead-exposed group were provided with normal diet and double-distilled water supplemented 200 mg/L of lead acetate. The rats in three selenium groups were similarly exposed to lead acetate and received a daily intragastric administration of, respectively, 2, 4, or 8 mg/kg Na2SeO3. The rats in control group consumed normal diet and double-distilled H2O. Twenty days after conception, 10 rats from each group were randomly selected for pathology studies. The remaining rats were provided with normal diet and double-distilled water, with or without selenium gavage, after delivery of their offspring.

**Measurements of serum lead and selenium levels, and reproductive hormones, and observations of genital tissue under electron microscope**

Twenty days after conception, blood was obtained from hearts of pregnant rats after intraperitoneal anesthesia with 2% pentobarbital sodium (30 mg/kg). Serum lead levels were measured with the WFX-120 graphite furnace spectrophotometer, and serum selenium levels were quantified with the AF-610A atomic fluorescence spectrometer. The follicle stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone were measured with respective radioimmunoassays.

In addition, uterine and ovarian tissues were extracted from the rats and fixed in 2.5% glutaraldehyde. Then, the tissues were rinsed with phosphate buffer (pH 7.2) and fixed with 1% osmium tetroxide for 1.5 hours. After ethanol dehydration, embedding, and ultrathin sectioning, ultrastructures of uterine and ovarian ultrastructures were observed under the Hitachi H-7500 transmission electron microscope.

**Growth and physiological development of the offspring**

The number of the offspring was recorded at birth. The offspring gender was distinguished via anogenital distance measurements using a Vernier caliper. Anogenital distance is defined as the distance between the frontmost edge of the anus and the backmost edge of the genitals. Any genital malformation was noted. Weights of the offspring were measured every 7 days (i.e., on days 0, 7, 14, 21, 28, and 35) after birth. Survival rates at days 4 and 21 were recorded. On day 21 after birth, the litter was separated by gender. Twenty rats (10 males and 10 females) were randomly selected from each group for pathology studies. Specifically, their reproductive organs were removed and weighed.
Then, 20 other rats (10 males and 10 females) were randomly selected from each group. Male rats were monitored for foreskin separation starting from day 35 after birth. Observations were continued until all of male rats exhibited foreskin separation. Vaginal openings of female rats were checked every morning at 9:00 starting from 27 days after birth until all female rats exhibited vaginal openings.

**Statistical Analysis**

Data are presented as mean ± SD. The groups were compared using the One-Way ANOVA or chi-square tests. The SPSS 12.0 (SPSS Inc., Chicago, IL, USA) statistical package was used for statistical analysis. Differences at $p < 0.05$ were considered statistically significant.

**Results**

**General condition, and serum levels of lead and selenium**

During the experiment, we did not observe abnormal behaviours or abnormal physical appearance of pregnant female rats exposed to lead. Specifically, we did not witness indifferent reactions, or extreme levels of excitement, changes in dynamicity, or presence of dry, yellow, or shedding fur. In addition, no delivery or nursing abnormalities (labour dystocia, stillbirth, or malformation) were observed.

Nevertheless, when we obtained serum specimens and determined the levels of lead, these were 100% higher in the lead-exposed animals compared with control animals (respectively, $122.162 ± 9.378$ vs. $60.317 ± 4.721 \mu g/L$, $p < 0.05$; Figure 1A). Supplementation with selenium dose-dependently decreased serum lead levels. Thus, low, intermediate, and high dose supplementation respectively reduced serum lead levels by 3.862%, 29.529%, and 51.853% (Figure 1A). The decrease in serum lead levels in the groups supplemented with intermediate and high doses of selenium was significant ($p < 0.05$ vs. lead group; Figure 1A). As expected, selenium supplementation dose-dependently increased selenium levels in serum (Figure 1B). Thereby, there was a reverse association between selenium and lead serum levels.

**Reproductive hormone levels**

Exposure to lead caused a significant ($p < 0.05$) drop in serum levels of follicle-stimulating hormone and estradiol (respectively, Figures 2A and 2D), and significant increases in serum levels of luteinizing hormone (Figure 2B), prolactin (Figure 2C), and progesterone (Figure 2E; all $p < 0.05$). Selenium supplementation normalized the levels of all tested reproductive hormones. Thus, follicle-stimulating hormone and oestradiol levels increased, whereas the levels of luteinizing hormone, prolactin, and progesterone decreased (Figures 2A-2E). These results demonstrated that protective effects of selenium supplementation occurred at the level of reproductive hormones.

**Uterine and ovarian morphologies**

We further observed uterine and ovarian morphologies to detect the damaging effects of lead exposure to the reproductive system. We documented the presence of moderate oedema and fusion disappearance in the mitochondrial arrangements in specimens of rats exposed to lead (Figure 3). Furthermore, rough endoplasmic reticulum expansion and degranulation were also evident (Figure 3). These changes

![Figure 1. Serum lead and selenium levels in pregnant rats.](image-url)
were also present in rats exposed to lead and supplemented with low dose of selenium (Figure 3). However, starting from the intermediate dose of selenium, the observed morphological changes were attenuated (Figure 3). Thus, the extent of oedema became less pronounced, and there was only partial fusion disappearance in the mitochondrial arrangements, as well as mild rough endoplasmic reticulum expansion and degranulation (Figure 3). Rats supplemented with high dose of selenium demonstrate no morphological changes (Figure 3). All of the above observations were applicable to uterine and ovarian tissues (respectively, A and B panels of Figure 3).

Sex differentiation in the offspring

Sex differentiation is influenced by genetics, hormonal regulation, and other factors. The mean anogenital distance was calculated in the next ex-
performed as a measure of sex differentiation. We observed no statistically significant differences in the mean anogenital distances among animal groups (Figure 4 A). Furthermore, there were no differences in the pups’ number among groups (Figure 4 B). Finally, we did not observe differences in the sex ratios (i.e., number of male vs. female offspring) (Figure 4 C). These observations excluded effects of lead exposure or selenium supplementation on the tested parameters of sex differentiation.

**Growth and development of the offspring**

Next, we evaluated offspring survival rates on days 4 and 21. Furthermore, since weight is one of the key indicators of the growth and development of the offspring, we measured the weights of the offspring at days 0, 7, 14, 21, 28, and 35.

While survival rates of the lead-exposed group tended to be lower on days 4 and 21, these differences did not reach statistical significance (Figure 5A). However, average weights of this group were
significantly lower on days 0-28 after birth (Figures 5 B and 5 C). After 35 days, no significant differences in weights existed among the groups. Rat weights, attenuated on days 0-28, were significantly enhanced by supplementation with high dose selenium (Figures 5 B and 5 C).

These observations indicate that exposure to lead during pregnancy markedly decreases the growth of the offspring, which is attenuated by selenium supplementation.

Reproductive organ development of the offspring

Development of reproductive organs affects sexual maturation and reproductive functioning of the offspring. We next studied how lead exposure, with or without selenium supplementation, affects reproductive organ development of the offspring. Specifically, we measured the weights of the testis and epididymides, or ovaries and uterus.

Lead exposure significantly \((p < 0.05\) for all observations; Figure 6) decreased the reproductive organ weights. Selenium supplementation dose-dependently attenuated these pathological changes, and significant differences were achieved by either intermediate or high dose supplementation (Figure 6), further highlighting protective effects of selenium.

Puberty onset in the offspring

Puberty onset is indicated by foreskin separation or vaginal opening. To test for this outcome, we recorded the number of days required for foreskin separation or vaginal opening.

Both foreskin separation and vaginal opening were significantly delayed in lead-exposed animals (respectively, 3.664 and 3.968 days; both \(p < 0.05\); Figure 7). Selenium supplementation, especially with intermediate and high dose, decreased the time required for foreskin separation (respectively, by 1.887 and 3.013 days, both \(p < 0.05\); Figure 7). In a similar manner, high dose selenium significantly decreased the number of days required for vaginal opening (decrease by 2.994 days; \(p < 0.05\); Figure 7). These results indicated that selenium supplementation attenuated deleterious effects of lead exposure on the onset of puberty in the offspring.

Discussion

Supplementation with selenium has been reported to benefit patients with the prostate, lung, colorectal, and bladder cancers12-14. Selenium supplementation can also promote resistance to viruses and maintain reproductive function15,16. Furthermore, it attenuates the damage to the kidneys, the liver, and other organs caused by lead exposure, and improves cognitive ability17-21.

Lead damages blood cells and affects the nervous, urinary, and immune systems22,23. Due to accumulation in the body, long-term exposure to low doses of lead can affect reproductive function24,25. Here we exposed SD rats to lead during pregnancy and tested potential protective effects of supplementation with different doses of selenium. Our observations indicated that gestational lead exposure damaged reproductive systems of pregnant rats and caused uterine and ovarian cell morphology changes, leading to changes in reproductive hormones. These toxic effects of lead were reduced by selenium supplementation.

Lead causes the damage to both pregnant rats and, by crossing the placental barrier and passing through breast milk, the offspring. Thus, it affects hormone secretion and organ development in the offspring26-29. Exposure to lead can also cause abnormal morphological changes and dysfunction of the reproductive system, including the oestrogen-like activity during sex differentiation in the offspring. Under normal circumstances, the anogenital distance of offspring is strictly regulated by endogenous sex hormones. Androgens ex-
selenium supplementation may be used to treat lead poisoning during pregnancy. These adverse effects are reverted or attenuated by selenium supplementation. Therefore, we demonstrate damages to the reproductive system. These adverse effects are reverted or attenuated by selenium supplementation. Therefore, selenium supplementation may be used to treat lead poisoning during pregnancy.

Conclusions

In a rat model of gestational lead exposure, we demonstrate damages to the reproductive system during pregnancy, and negative modulation of growth and reproductive system development of the offspring. These adverse effects are reverted or attenuated by selenium supplementation. Therefore, selenium supplementation may be used to treat lead poisoning during pregnancy.

Conflicts of interest

The Authors declare that there are no conflicts of interest.

Reference