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Research paper

Therapeutic effect of spermatogonial stem cell on testicular damage caused by lead in rats

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ABSTRACT

Objective: To investigate the possible therapeutic effect of spermatogonial stem cells (SSCs) on lead-induced apoptosis and consequently infertility in adult male rats.

Materials and methods: Sixty-six Sprague Dawley adult male rats were divided into three groups: control group, lead (Pb) acetate exposed group received (20mg Pb/kg) for 3 weeks, and SSCs treated group. Each group included twenty-two rats. Serum testosterone level, 3 beta-hydroxysteroid dehydrogenase (3β-HSD), 17 beta-hydroxysteroid dehydrogenase (17β-HSD), proliferating cell nuclear antigen (PCNA) genes expression by RT-PCR, caspase 3 expression by immunohistochemistry and testicular histological findings were tested.

Results: Pb acetate exposed rats showed a significant decrease in the epididymal sperm count, motility, viability, serum testosterone level and testicular expression of 3β-HSD, 17β-HSD and PCNA compared to the control group, while treatment with SSCs attenuated Pb acetate induced decrease for these variables. Moreover, the increasing apoptosis of germinal cells as well as the high expression of caspase-3 induced by Pb acetate was reduced by SSCs treatment.

Conclusion: SSCs exhibited therapeutic effect on reproductive system by inhibiting Pb-induced excessive cell apoptosis.

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1. Introduction

Although lead (Pb) is a useful metal in life, used in modern industries and agriculture, as making some types of glass, lead-acid storage batteries, insecticides, chrome yellow paint and the production of rubber. It is one of the most toxic heavy metals for body and its poisoning is known as an important public health problem (Alfano and Petit, 1981; Altmann et al., 1993; Hsu and Guo, 2002; Patrick, 2006; Woolf et al., 2007; Xu et al., 2006). The toxic effects of lead can be manifested in various organs and the male reproductive organ is clearly an important target (Alexander et al., 1996).

Common effects of lead seen in men include: reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function and changes of serum testosterone levels (Flora et al., 2011). Considerable evidence exists that toxic effects of lead have been demonstrated to be closely associated with a relatively high rate of apoptosis (Wang et al., 2006). Adhikari et al. (2001) reported that Pb could induce apoptosis of the germ cells within the seminiferous tubules.

Apoptosis, a gene-regulated process, is a normal cellular response to the development, differentiation and environmental stress (Heidmets et al., 2006; Silbergeld, 2003). Testicular apoptosis serves to deplete excess germ cells and remove the abnormal spermatozoa during the normal spermatogenesis as it eliminates 75% of germ cells before they become fully mature (Martincic et al., 2001; Shaha et al., 2010).

In this way, testicular apoptosis monitors germ cell population according to the support capacity of Sertoli cells and it is essential for maintaining the testicular homeostasis during spermatogenesis (Shaha et al., 2010). Inappropriate apoptosis, resulting from impaired regulation or improper activation, can affect spermatogenesis and even lead to infertility (Lizama et al., 2007; Martincic et al., 2001; Print and Loveland, 2000).

Spermatogenesis is a cyclic, well-organized process that generates ~100 million sperms each day in adult males (Dadoune, 2007; DeRooij, 1998; Sharpe, 1994). It starts with a small number of spermatogonial stem cells (SSCs) which undergo self-renewal division, proliferation, and differentiation to produce sperms.

A single SSC can produce two stem cells, by asymmetric division they can produce one stem cell and one differentiating cell, or undergo

Abbreviations: PCR, polymerase chain reaction; 3β HSD, 3 beta hydroxyl steroid dehydrogenase enzyme; SCC, spermatogonial stem cell; FBS, fetal bovine serum.

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asymmetric/differentiating cell division that results in two differentiated cells. Most of the diploid germ cells and differentiating spermatogonia undergoes several rounds of mitotic divisions before they enter into meiotic phase and later differentiate to become haploid spermatids that subsequently become mature spermatozoa in about 35 days in the mice, 49 days in rats and 64 days in the human (Brinster, 2002; Brinster, 2007; Campion et al., 2013).

The proliferating cell nuclear antigen (PCNA) is a 36 kDa auxiliary protein to DNA polymerase-δ that is required during DNA replication and nucleotide excision repair. It also interacts with cyclin dependant kinases, which are associated with cell cycle control (Godlewski et al., 1999). PCNA has been used extensively in the identification of proliferating spermatogonia and spermatocytes in a number of species, notably human rhesus and cynomolgus monkeys and rats (Sheba et al., 2005).

Although adverse effects of lead compounds on the testis have long been studied (Oliveira et al., 2006), effective drugs reversing the toxicity of lead on male reproduction are still scarce and insufficient. In this regard, the aim of the present study was to investigate the possible therapeutic effects of SSCs on lead-induced apoptosis and consequently infertility.

2. Materials and methods

2.1. Preparation of donor cells and transplantation analysis

Donor testes prepared according to Nayernia et al. (2004). The whole testes were isolated from five Sprague Dawley rats (the age between 5–10 d), were washed in phosphate-buffered saline (PBS), and were put in 5 mL of collagenase/Hanks' balanced salt solution (HBSS) (pH 7.4) in 60-mm sterile Petri dish. Then they were incubated for 10–15 min at 37 °C with gentle agitation in a rocker platform until the tubules separated. The tubules were poured off and transferred to 15 mL centrifugation tube and were centrifuged at 4 °C at 650 ×g for 5 min. The supernatant was added to 5 mL of HBSS, and was centrifuged again at 4 °C at 650 ×g for 5 min. Then 2 mL of dispase solution was added and stirred slowly at 37 °C until the tissue was sufficiently dissolved (30 min). Dispersion of the tubule cells was hastened by pipetting and gentle agitation. Cells were pelleted by centrifugation at 16 °C at 650 ×g for 5 min. The pellet was resuspended in 400 mL Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) at a concentration of $(2–3) \times 10^7$ cells/mL. Trypan blue at concentration (0.4%) was added to the cell suspension. The cells were maintained for 5 h at 4 °C until transplantation. The cells were transplanted into the seminiferous tubules by inserting a needle through the efferent ductules of recipient rats outside of the testis and passing the needle into the rete testis (Nayernia et al., 2004). After entering the rete, the pressure was increased very slowly in the injection tube until the cell suspension fills the rete and flows into the tubules. About 10–20 μL of cell suspension is required to fill one testis.

2.2. Animals and experimental design

The experiment was performed on Sprague Dawley adult male rats weighing approximately 125–135 g obtained from the Faculty of Veterinary Medicine at Zagazig University. The animals were kept in plastic cages with 12-hr alternating light dark cycle. All rats were acclimatized for 4 weeks prior to treatment. Eight-weeks-old rats, equivalent to humans at the age of young adulthood (Quinn, 2005), were assigned to three groups, each comprising 22 rats: (1) control group: animals served as negative controls and received tap water alone; (2) Pb acetate exposed group: animals were treated with Pb acetate (20 mg/kg), by oral gavage daily for 21 days. The Pb acetate dose was derived from previously investigation on rat toxic models (Wang et al., 2006). (3) SSCs treated group: Pb acetate exposed animals (20 mg/kg for 21 days) then on the 22nd day, stem cells were transplanted in both testes. All the rats were anaesthetized with ether and killed by cervical dislocation on the 70th test day from the beginning of exposure to lead acetate.

We started with 25 rats in each group and the success rate in our study was 96% in group 1 and 3 while it was 88% in group 2. In order to make the number of rats constant in all groups, we excluded two rats from each of the first and third groups.

i.e. This study were according to the guidelines set forth by the National Institutes of Health (USA) which was approved by our Institute Ethical Committee.

2.3. Body and testis weights

The testes were removed and weighed. Finally, the relative testis weights were calculated by dividing left testis weight by body weight and then multiplying it by 100 (left testis weight/body weight × 100).

2.4. Sperms isolation

Epididymis and vas deferens were separated from testes and were put into 2 mL of pre-warmed PBS, pH 7.4. Spermatozoa were allowed to diffuse after the epididymal tubule was pierced with a scalpel blade, and the spermatozoon was forced out of the vas deferens with fine forceps by putting pressure on the lower region of the cauda epididymidis and "walking" the forceps down the vas deferens. The dish was shaken gently and after 5 min of dispersion, an aliquot of spermatozoa was used for sperm count, motility and viability (Eliasson, 1997).

2.5. Sperms count, motility and viability

An aliquot of spermatozoa was diluted 1:100 with fixative (10% formalin in PBS, pH 7.4) and counted using a haemocytometer. Epididymal sperms motility was also evaluated in the PBS, pH 7.4. 50 μL aliquot was diluted 20 times in PBS at 37 °C, transferred to a glass slide, a random microscopic field was chosen and the spermatozoon was classified as motile or immotile. Sperms motility was expressed as the percentage of motile sperms per field. Sperms viability was checked by eosin–nigrosin staining. 20 μL aliquot was mixed with two drops of 1% eosin Y. After 30 s, three drops of 10% nigrosin were added and were mixed well. A smear was made by placing a drop of mixture on a clean glass slide and was allowed to air dry. The slide was examined under microscope. Pink-stained dead sperms were differentiated from unstained live sperms, and their numbers were recorded. For sperm abnormality, sperm smears were dried overnight and then stained with 10% Giemsa (diluted with Sorenson's buffer, pH 7.0) for 1 h and subsequently observed.

The sperms count, morphologically abnormal sperms, sperms motility and sperms viability were calculated and analyzed according to the methods of Sokol (1990), Wyrobek and Bruce (1975) and Wadi and Ahmad (1999). The live-sperms rate of around 40–50% was considered as the standard for a successful model of testicular damage.

2.6. Assay of serum testosterone concentration

Serum concentration of testosterone was measured with commercially available ELISA Kit (DRG, USA).

2.7. Testicular metal analysis

2 g of homogenized samples were taken into 50 mL quartz crucible and dried in an oven at 120 °C overnight until the samples were thoroughly dry then the temperature of the oven was raised to 300 °C for 8 h. Next day, the samples were removed from the oven and were cooled to room temperature. 1 mL concentrated nitric acid was added and the volume was adjusted to 25 mL with deionized water. Lead concentrations in samples were determined by atomic absorption

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