Structural Changes of the Testis and Changes in Semen Quality Parameters Caused by Intraperitoneal and Peroral Administration of Selenium in Rats

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Abstract
The aim of this study was to find the structural changes in the testis and semen quality parameters of rat after a single intraperitoneal and repeated peroral selenium administration. Rats were killed 36 hours following the intraperitoneal administration of selenium selenite (2 $mg.kg^{-1}$ b.w.; 98% purity) and after 90 days of the peroral repeated administration of selenium in drinking water (5 $mg.l^{-1}$). Testis samples were evaluated by histological and morphometrical methods in light microscopy. Evaluation of semen samples were examined with CASA method. 36 hours after the selenium i.p. administration, damage of cellular associations, release of necrotised epithelial cells to tubule lumen and fibrotisation and extension of interstitium were observed. Morphometry methods have shown the reduction of seminiferous epithelium volume ($P<0.001$), extension of interstitium ($P<0.001$) and increased area of intraepithelial spaces ($P<0.01$). In p.o. group similar but more intense changes were noted; in addition, occasionall degeneration of seminiferous tubuli and rarely total damage in histoarchitecture of seminiferous epithelium were observed. CASA analysis revealed significant decrease in all parameters except the concentration of spermatozoa. Additionally, we suppose that p.o. dose 5 $mg.l^{-1}$ sodium selenite in drinking water is minimum lethal dose level for young rats. Selenium after i.p. and p.o. administration causes damage of seminiferous epithelium and interstitium. It leads to changes in relative proportion of functional tissues of the testis. Reduced spermatogenesis and harmful effects in semen parameters are characteristic especially for peroral repeated (subchronic) administration. These changes are time- and dose-dependent. In both dosage methods subfertility or infertility can appear.

Keywords: CASA, histology, morphometry, rat, selenium, sperm, testis

1. Introduction

In recent years interest of scientific teams and then also public about essential microelement selenium greatly increased. Its effects are in the environment as well as in a living organism linked mostly to its biochemical form, concentration and periodicity of exposure. These parameters determine the beneficial or harmful effects of selenium. In endemic or contaminated areas (outside the optimal levels) of environment, selenium may act as limiting trace element or contaminant, both with harmful effects for living organisms of the ecosystem. The same effect for organisms has selenium in inappropriately diet or feed ration.

In human or animal organism it is essential microelement in a physiologically optimal level with many functions. Homeostatic regulation of selenium is considerably limited. Deficit leads to deficit symptoms and excess lead to toxic effects. Well known is its antioxidant, prooxidant and metabolic effects [1]. Selenoproteins,

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selenoenzymes and other biochemical forms participate in the antioxidant protection system of cells and their organelles against free radicals damage and the associated/linked diseases [2]. Some forms of selenium can act as prooxidant on various types of cancerous cells and this is considered as the way of their protective function in prevention of some types of cancers and the overall anticarcinogenic activity [3-7]. Selenium participates on deiodination of thyreoidal hormones and thereby indirectly affects energy metabolism [8-9]. Selenium significantly impacts sensitive metabolic regulations of male reproductive system. In the form of selenoenzymes and selenoproteins (PHGPx/GPx4, selenoprotein P and others) is essential for testicular development, spermatogenesis and sperm functions [10-14]. In idiopathic infertile men, positive correlations between selenium concentration and plasma testosterone were detected [13]. Many authors suggest the use of selenium alone or in combination with other antioxidants for fertility promotion and treat some forms of male sub- or infertility [13,15,16]. On the other hand, less known are effects of excess of selenium on the male reproductive system, complex mechanism of actions and relation between the results of different types of experiments. Kaushal and Bansal [17] reported prooxidant effects of selenium on germ cells after administration of an excess of sodium selenite to the mouse in their feed.

The aim of experiment was to describe and evaluate structural changes in the testis and sperm quality parameters of the rat after single intraperitoneal and repeated peroral administration of selenium.

2. Materials and methods

Experimental animals and design

30 Wistar rats were randomly divided to three groups: control group (C), group with single intraperitoneal administration of selenium (IP) and group with repeated peroral (subchronic) administration of selenium (PO). Age of rats at the beginning of the experiment was: 120 days for groups C and IP and 30 days for PO group. Rats of IP group were injected with selenium as sodium selenite 2 $mg.kg^{-1}$ b.w. (Na_2SeO_3; 98% purity; Reachem, Bratislava) in saline solution. PO rats recieved same sodium selenite in their drinking water (5 $mg.l^{-1}$ for 90 days) with free access. At the end of experiment rats of all group were anesthetized (ethyl ether) and humanely killed (IP group 36 hours after selenium administration and PO group after 90 days of Se administration). Anatomical dissection was performed and samples of testicles were fixed in modified Davidson's solution [18]. Animals were housed individually in plastic cages with wood shavings bedding. In the experimental laboratory basic requirements of environmental conditions (temperature 20 to 22°C, air humidity 55±10%, 12 hours dark/light regime) and unlimited access to drinking water and feed (M3, Máchal, Czech republic) were maintained according to Government regulation no. 289/2003. Experiment was performed in an approved experimental laboratory SK PC 50004 SPU in Nitra, Slovak republic.

Histological and morphometrical evaluation

Samples of testis were stained with hematoxylin-eosin and were examined using the light microscopy (Nikon Eclipse E600, Japan). Morphometrical evaluation of photomicrographs was performed using computerized evaluation morphometric software M.I.S. Quick Photo with assistance of microscope Olympus AX 70 (Olympus, Japan) and further evaluated with quantitative morphometrical method modified by Uhrín and Kulíšek [19]. Testicular weight (g), mean seminiferous tubule diameter (μm), relative volume of testicular structures (seminiferous epithelium, intraepithelial empty spaces, tubule lumen, interstitial tissue, blood vessels) (%) were determined.

Evaluation of semen quality parameters

Epididymis was taken during anatomical dissection immediately after killing the animals and semen samples were taken. Samples were diluted with saline solution (20 μl) kept at the 37°C. The samples were placed to 10μm deep Makler counting chamber (Sefi-Medical instruments, Israel) and then were evaluated at least 8 representative fields of view with software SpermVision (Minitüb, Tiefenbach, Germany) and

microscope Olympus BX 51 (Olympus, Japan). Following parameters were evaluated:

- spermatozoa concentration
- % of motile spermatozoa
- % of progresive motile spermatozoa
- DAP - distance average path (μm)
- DCL - distance curved line
- DSL - distance straight line
- VAP - velocity average path ($\mu m.s^{-1}$)
- VCL - velocity curved line
- VSL - velocity straight line
- STR - straightness (VSL/VAP)
- LIN - linearity (VSL/VCL)
- WOB - wobble (VAP/VCL)
- ALH - amplitude of lateral head displacement ($\mu m.s^{-1}$)
- BCF - beat cross frequency (Hz)

Statistical evaluation

Results were presented as mean±standard deviation (x±SD). For statistical evaluation of results one-way analysis of variance (one-way ANOVA) and Software Statgraphics Centurion XV with alpha level set as $\alpha = 0.05$ was used.

3. Results and discussion

Photomicrographs of control group testis tissue have shown normal shape and histological structure. The space between seminiferous tubuli was filled by condensed interstitial tissue. The outer edge of the tubuli perimeter was closely lined with basement membrane and towards the lumen in irregular layers there were added germ cells in different stages of development, between which there were sketched Sertoli cells with characteristic nucleus. Depending on the stage of spermatogenic cycle at the top of apical Sertoli cells cytoplasm there were embedded spermatids. Lumens of seminiferous tubuli were filled with masses of released spermatozoa. In the interstitial tissue, blood vessels with fundamentally different diameters surrounded by numerous of Leydig cells and sometimes typical filaments structure of interstitial tissue were well seen. Weight of testis and diameter of semniniferous tubuli are shown in Table 1, the relative volume of the testis is presented in table 2 and semen quality parameters are shown in table 3.

Table 1. Testis weight (g) and diameter of seminiferous tubuli (μm)

Group	Testicular weight	Seminiferous tubuli diameter
	x±*SD*	
Control	1.45±0.25	250.37±15.09
Selenium IP	1.44±0.08	255.52±15.94
Selenium PO	1.41±0.16	228.91±38.00

x-mean, *SD*-standard deviation

Excess of selenium in the body causes disruption of homeostasis which leads to acute stress reaction [20,21] with characteristic result – increased production of free radicals [20-22]. This overproduction of free radicals causes damage of cells and their structures [17,22-24], increased lipid peroxidation [22,25], damage of DNA [22,24-25] with the subsequent histo- and physiopathological manifestations [17,22,27]. Changes induced by excess of selenium are dose- (intensity of stress response) and time-dependent (duration of action of free radicals) [1]. These mechanisms seem to be a link between the toxic effects of many essential elements administered in excess.

After a single intraperitoneal administration of 2 $mg.kg^{-1}$ sodium selenite, changes in weight of testis or diameter of seminiferous tubuli were not observed (Table 1). Frequently observed histopathological change was damage of cell associations which consequently leads to ruptures of seminiferous epithelium, or intraepithelial spaces in areas of nercotized germ cells released from epithelium to the tubule lumen (Figure 2). Kaushal and Bansal [22] observed apoptosis of germ cells in male mouse after the administration of selenium in excess which corresponds with our findings. Damage of condensed structure of interstitium was also observed. Naked and released reticular and collagen fibers in the interstitium formed relaxed fiber networks compared to control (fibrotisation) (Figure 2). Fibrotisation and extension of interstitium are possible signs of interstitial inflammation as result of acute stress response. Separation of the basement membrane from the epithelium was rarely observed. The toxicity of selenium causes also its prooxidant properties [28]. Nebbia et al. [29] noted increases activity of lactate dehydrogenase and β-glucuronidase in testis tissue

after administration 4, 8 or 16 ppm of sodium selenite in drinking water to rats which indicates tissue damage and breakdown. Their findings correspond with our histopathological findings in seminiferous epithelium and interstitium. Reduction in volume of seminiferous epithelium (P<0.001), free spaces in the epithelium (P<0.01), to the detriment of extension of interstitium (P<0.001) and insignificant extension of lumen of seminiferous tubuli and blood vessels were noted (Table 2).

Table 2. Relative volume of testis structures (%)

	Seminiferous epithelium	Intraepithelial spaces	Tubule lumen	Interstitium	Blood vessels
Group			*x* ± *SD*		
Control	64.82±2.90	0.38±0.74	23.58±3.03	10.93±2.03	0.29±0.27
Selenium IP	55.74±5.10***	2.07±1.56**	25.33±3.01	16.10±3.18***	0.76±0.82
Selenium PO	56.89±2.89***	1.49±1.21*	18.24±7.38	22.89±6.04***	0.49±0.39

x-mean, *SD*-standard deviation; * P<0.05; ** P<0.01;*** P<0.001

The most important change in the proportions of morphometrically evaluated components of testis was between damaged seminiferous epithelium and interstitial tissue. These changes might be potentially caused (in view of the short duration of treatment) by effects of free radicals (direct prooxidative effect of selenium and selenium-mediated stress reaction) in addition with direct effects of stress hormones (epinephrine, cortisol) to the structure and functions of testis. Evaluation of semen quality parameters has shown only insignificant changes. The most expressive change was increase in concentration (0.39±0.71 vs. 0.17±0.12), motility (39±23 vs. 29.66±15.88) and progressive motility (19.45±14.92 vs. 13.82±10.46), other parameters when compared with the control group (Table 3). In PO group after subchronic peroral administration of sodium selenite (5 $mg.l^{-1}$/90 days) one of rats died. Palmer and Olson [31] not recorded mortality following administration 2 and 3 ppm of sodium selenite to adolescent rats in drinking water. Increasing mortality was observed after administration of 6 and 8 ppm sodium selenite [31]. The dose used in our experiment (5$mg.l^{-1}$ of drinking water/90 days) might be potentially the minimum lethal dose level for adolescent rats for the given route of administration. Weight of testis and diameter of seminiferous tubuli were not significantly decreased (Table 1). Similar but more intense histopathological changes than were noted in IP group were observed. Histoarchitecture of semniniferous tubuli was damaged, in the epithelium less or more extensive free spaces in the areas of released necrotized germ cells released to the tubule lumen were visible. Additionally some parts of seminiferous epithelium were partly to completely degenerated - some stages of spermatogenic cells (from spermatogonia to late spermatids) was partly to totally undeveloped and in these places there was only Sertoli cells with their connections (Figure 3). In many places of seminiferous tubuli there was separating basement membrane with or without few germ cells. Morphometrically, the same statistically significant changes like in IP group was observed, except insignificantly decreased volume of interstitial tissue (Table 2). Selenium in excess causes oxidative stress in testicular germ cells, damage of RNA and DNA, reduction of the expression of genes required for synthesis of proteins essential for spermatogenesis [17,26]. Optimal production of spermatozoa in rats occurs first in the 45^{th} postnatal days and optimal production is achieved first in the 75^{th} days [31] consequently subchronic administration of selenium in our design of experiment potentionally influenced also testicular development. Evidence of this hypothesis might be partly or completely degenerated seminiferous tubuli. All evaluated parameters of semen quality in PO group were also significantly decreased, except insignificant decrease of spermatozoa concentration (Table 3). All parameters describing

motility are closely related to sperm mitochondrial section, site of energy production. Kaur and Parshad [25] reported morphological damage of mitochondrial section of spermatozoa after the peroral feeding of 4 ppm sodium selenite to the rats which supports our findings of reduced parameters of motility. These data suggest that the mitochondrial section might be directly or indirectly the target site of spermatozoon damage caused by selenium.

Table 3. Semen quality parameters

Group	Control	Selenium IP	Selenium PO
Parameters			*x±SD*
Concentration	0.17±0.12	0.39±0.71	0.12±0.10
%Motile	29.66±15.88	39±23	12.16±6.61**
%Progressive	13.82±10.46	19.45±14.92	3.87±3.91*
DAP	21.01±4.58	21.70±5.00	8.63±7.68**
DCL	33.78±8.22	33.71±8.11	13.54±10.75**
DSL	16.32±2.89	16.87±3.58	6.99±6.10**
VAP	53.39±12.65	54.46±12.30	21.16±18.59**
VCL	84.87±21.58	84.32±19.85	33.05±25.81**
VSL	41.42±7.85	42.31±8.67	17.29±14.95**
STR	0.79±0.05	0.78±0.05	0.40±0.30**
LIN	0.52±0.08	0.53±0.05	0.28±0.22*
WOB	0.65±0.08	0.66±0.03	0.33±0.27**
ALH	5.49±1.13	5.54±0.95	2.49±2.12*
BCF	17.90±1.75	17.92±3.08	7.66±6.27***

x – mean; *SD* – standard deviation; *P<0.05; **P<0.01; ***P<0.001

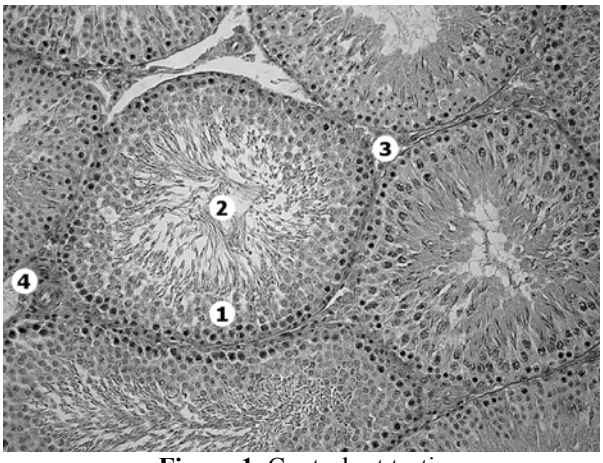

Figure 1. Control rat testis
1-seminiferous epithelium; 2-tubule lumen with spermatozoa; 3-interstitial tissue with Leydig cells; 4-blood vessels

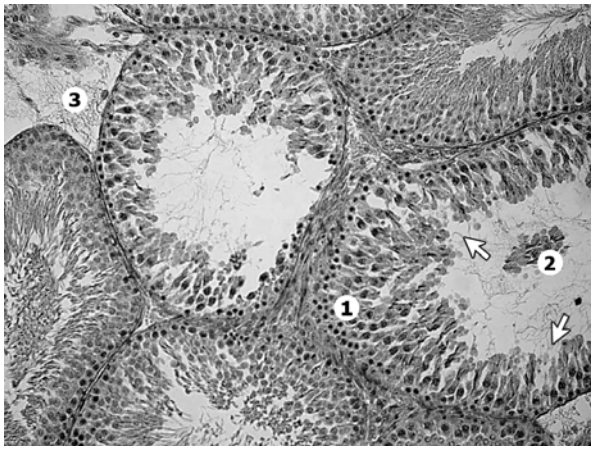

Figure 2. Rat testis after i.p. selenium administration
1-seminiferous epithelium; 2-tubule lumen with released germ cells; 3-fibrotised interstitium with net of reticular and collagen fibers; arrows-intraepithelial spaces – site of release germ cells

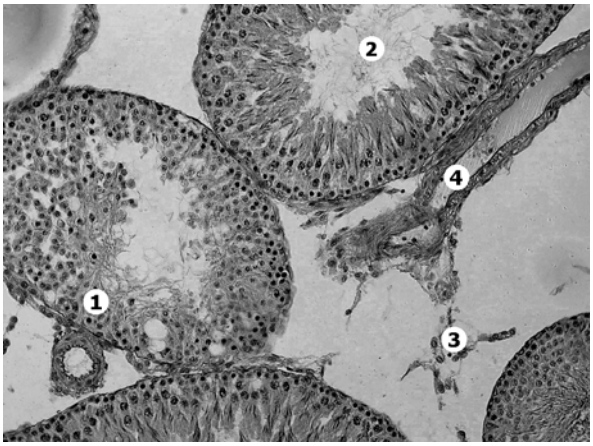

Figure 3. Rat testis after p.o. selenium administration
1-site of partly degenerated-undeveloped seminiferous epithelium only with spermatogonia; 2-tubule lumen with sperm; 3-interstitial tissue with Leydig cells; 4-blood vessel (longitudinal section)

4. Conclusions

Dose of sodium selenite 5 $mg.kg^{-1}$ administered to adolescent rats in drinking water with free access is potentionally the minimum lethal dose level for this design of experiment. Selenium causes damage of cells associations and cell necrosis in seminiferous epithelium in both experimental groups. Damage of condensed structure of interstitial tissue was characteristic for IP group, degeneration of seminiferous tubuli structure for PO group. The same trends were described in morphometrical evaluation in both experimental groups, volume of seminiferous epithelium was reduced and volumes of interstitial tissue and free

spaces of epithelium were increased. Changes in semen quality parameters (parameters of motility) have been significant only in PO group and appear to be time dependent. In both groups subfertility or infertility can appear. For complete explantation of the mechanism of selenium toxicity to male reproduction system further complex studies are needed.

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