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A stereological and histopathological study of the effects of exposure of male rat testes to mercury vapor

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Abstract

Mercury is ubiquitous in the environment; it is an occupational pollutant and a potential toxicant. We investigated the effects of exposure of rat testes to mercury vapor (Hg^0). Twelve male rats were divided into two groups of six: the rats of the Hg^0 group were exposed to mercury (1 mg/m^3/day) in a chamber for six weeks; the control group rats were housed under the same conditions without exposure to Hg^0. After the experimental period, the testes were removed, sections of testis were evaluated histopathologically after hematoxylin and eosin staining, and stereologically using the Cavalieri principle and optical fractionator methods. We found significant decreases in the total volume of testis, diameters of seminiferous tubules and total volume of seminiferous tubules. Significant decreases were detected in the numbers of Sertoli cells, spermatogonia, spermatocytes and spermatids of the Hg^0 group compared to the control group. In the Hg^0 exposed group, spermatogenic cells were degenerated and seminiferous tubules were atrophied.

Key words: histopathology, mercury vapor, rat, stereology, testis

Mercury (Hg) is found naturally in the environment in both organic and inorganic forms (Schuster et al. 2002); it commonly is associated with industry and gold mining (Grandjean et al. 1999). Hg causes tissue damage by increasing production of reactive oxygen species (Stohs and Bagchi 1995, Stacchiotti et al. 2009, Wu et al. 2011, Ercal et al. 2001). The neurotoxic effects of low doses of Hg have been demonstrated for fetuses (Marsh et al. 1995), infants (Grandjean et al. 1998) and adults (Sørensen et al. 2000, Yokoo et al. 2003, Tang and Li 2006).

Hg is oxidized after inhalation and converted to the divalent Hg^0 form, which is toxic to many tissues (Clarkson 1997) including the cardiovascular system (Alissa and Ferns 2011), pancreatic beta cells (Chen et al. 2010), female (Davis et al. 2001, Apostoli and Catalani 2011) and male reproductive system (Martinez et al. 2014).

The literature contains few reports concerning the effects of mercury compounds on the male reproductive system (Barregard et al. 1999, Keck et al. 1993). It has been shown that exposure to mercuric chloride causes increased free radical formation, which predisposes the testis to both structural and functional abnormalities (Boujbiha et al. 2009). Also, mercury exposure decreases the number of sperm cells (Orisakwe et al. 2001 and Homma-Takeda et al. 2001) and a correlation has been found between decreased fertility and $HgCl_2$ exposure (Lee and Dixon 1975). Moreover, DNA breaks were found in spermatozoa following Hg exposure (Arabi and Heydarnejad 2007). Young's syndrome, which is associated with obstruction of the upper epididymis in childhood, can result from Hg intoxication (Hendry et al. 1993). Hg also can accumulate in the lysosomes of

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the testicular epithelium as pigment granules (Eto et al. 1997).

We found no reports in the literature that used histology or stereology to study the effects of Hg^0 on the testis. Therefore, we investigated whether exposure to Hg^0 causes deleterious changes in testicular structure.

Material and methods

Animals

We obtained ethical approval for our study from the Experimental Research and Application Center of Atatürk University. We used 12 8 − 10-week-old 200 g adult male Sprague-Dawley rats obtained from the Experiment Animals Research and Application Center of Atatürk University. The animals were divided randomly into control (untreated) and experimental (Hg^0 exposed) groups (n = 6, each group). The Hg^0 group was exposed to 1 mg/m^3/day Hg^0 in a closed chamber for 6 weeks (Fig. 1). Hg^0 was generated by passing HEPA-filtered, charcoal-scrubbed, and temperature and humidity controlled air through a chamber containing 10–20 g of metallic Hg. The resulting Hg^0 was delivered to the exposure chamber at a controlled rate using mass flow controllers. Air exiting the chamber was passed through a HNO_3 solution to neutralize the Hg. Exposure concentrations in the chamber were analyzed once each hour using a Jerome model431-X Hg analyzer (Arizona Instruments, Phoenix, AZ) that is specific for Hg^0. Animals were exposed to Hg^0 between 8 AM and 5 PM each day. The control group was housed under the same conditions without exposure to Hg^0. During the experiment, the rats were housed under standardized conditions of 12 h light:12 h dark cycle, 22 ± 2° C and 50 ± 5% humidity. All animals had access to food and water *ad libitum*. After six weeks, rats in both groups were anesthetized by inhalation of 2 − 3% sevoflurane (Sevorane® Liquid 250 ml; Abbott, Istanbul, Turkey) in 100% oxygen. The testes were removed after perfusion of the animals with 10% neutral buffered formalin.

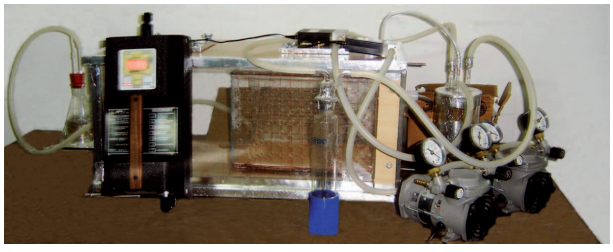

Fig. 1. Closed chamber for exposing rats to Hg^0.

Histology

All testes were fixed in 10% neutral buffered formalin, dehydrated through a graded alcohol series, cleared in xylene and infiltrated with paraffin for embedment. Sections of each testis were cut in a transverse plane at 5 µm using a rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany). Sections were mounted on slides, re-hydrated through descending concentrations of alcohol and stained with hematoxylin and eosin (H & E) (Kumtepe et al. 2010). We used the stained sections for examination by light microscopy and for stereological calculation of seminiferous tubule diameter, testicular volume and number of spermatogenic cells.

Stereology

Estimation of volumes of testis and seminiferous tubules

Serial sections obtained from each testis block were used to determine the volumes of structures using the Cavalieri principle (Altunkaynak et al. 2012a,b). According to the Cavalieri principle, the surface area of the biological object of interest can be defined by a specific point counting grid. The point densities of the point counting grid was designed based on pilot studies in which the point density of the point counting grids were designed to hit a minimum of 1,000 points per region of interest (testis and seminiferous tubules) for each animal; this was sufficient to obtain a significant coefficient of error (CE) (Dursun et al. 2010). After scanning the images and hitting points on them (Fig. 2a, b), the areas of the testis and seminiferous tubules were calculated separately. The value of measured surface area then was multiplied by the section thickness (t) and volumetric results were obtained (Fig. 2a). The volumes of the testis and seminiferous tubules were calculated using the following formula (Altunkaynak and Altunkaynak 2007, Altunkaynak et al. 2008):

$$V(total) = t \cdot \Sigma A$$

where "t" is the thickness of the slice (including intervals) and ΣA is the total sectional area of the testis sample sections. The term ΣA is equal to:

$$\Sigma A = a(p).\Sigma P$$

Where a(p) is the interval point area and ΣP is number of points hitting on favorable areas in the sections. The CE and coefficient of variation (CV) were calculated according to previously published formulas (Altunkaynak et al. 2013).

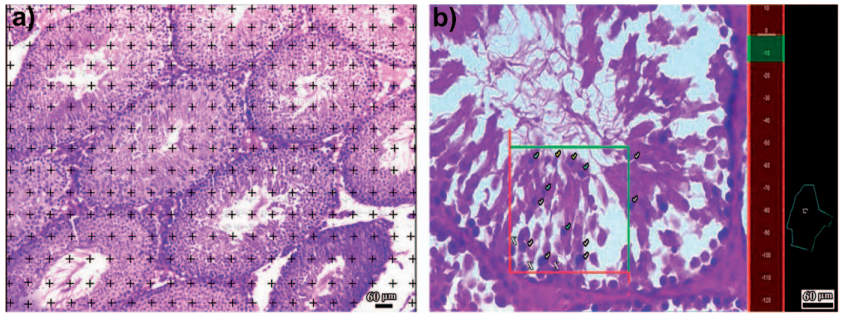

Fig. 2. Stereological procedures for applying the Cavalieri principle (a) and optical fractionator method (b).

Estimation of total numbers of spermatogonia, spermatocytes, spermatids and Sertoli cells

Cells of interest were counted using the optical fractionator method. The optical fractionator approach is the most frequently used method for estimating particle numbers (Gundersen 1986). Only nuclei within the optical disector frame were considered countable (Gundersen and Jensen 1987). The sampling and counting program was determined by a pilot study. The first section in the series to be analyzed was selected from the first six sections and every successive 5th section was collected from the series, which created a 1/6 section-sampling fraction (ssf). Approximately 15 – 20 sections from each testis are known to be adequate for estimating the number of objects using the optical fractionator method for cell counting (Gundersen and Jensen 1987, Tunc et al. 2006). Sampled sections were collected on gelatin-formaldehyde coated slides and stained with H & E. A stereology workstation consisting of a modified light microscope with motorized stage (Stereoinvestigator 9.0; MicroBrieldField, Colchester, CT) was used for cell counting. The spermatogonia, spermatocytes, spermatids and Sertoli cells were counted. The total numbers of the cells were calculated from the number of counted cells and the sampling probability (Gundersen, 1986). The total number of testicular cells was calculated by:

$$N = \Sigma Q \cdot \frac{1}{ssf} \cdot \frac{1}{asf} \cdot \frac{1}{tsf}$$

Where N = total number of testicular cells, ΣQ = total disector particle number, asf = area of sampling fraction and tsf = thickness of sampling fraction.

The precision of the calculations was determined as described by Gundersen and Jensen (1987) for CV and CE. The CE and CV of the sampling procedure was validated by a pilot study as described above (CE ≤ 10%) (Altunkaynak et al. 2011, Kıvrak et al. 2013, Yurt et al. 2013).

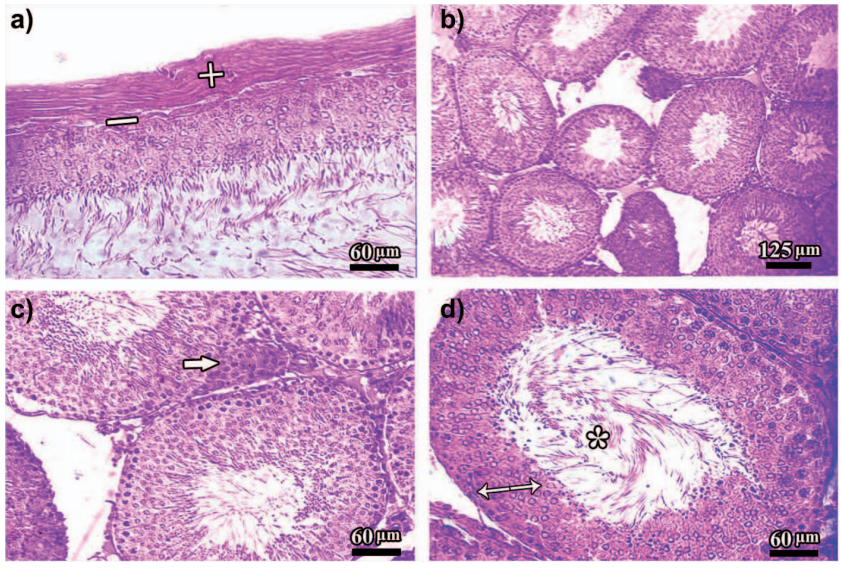

Fig. 3. Control group images (a – d). (+) tunica vaginalis; (–) tunica albuginea; arrow, Leydig cells; double-headed arrow, seminiferous epithelium; asterisk, lumen of seminiferous tubule.

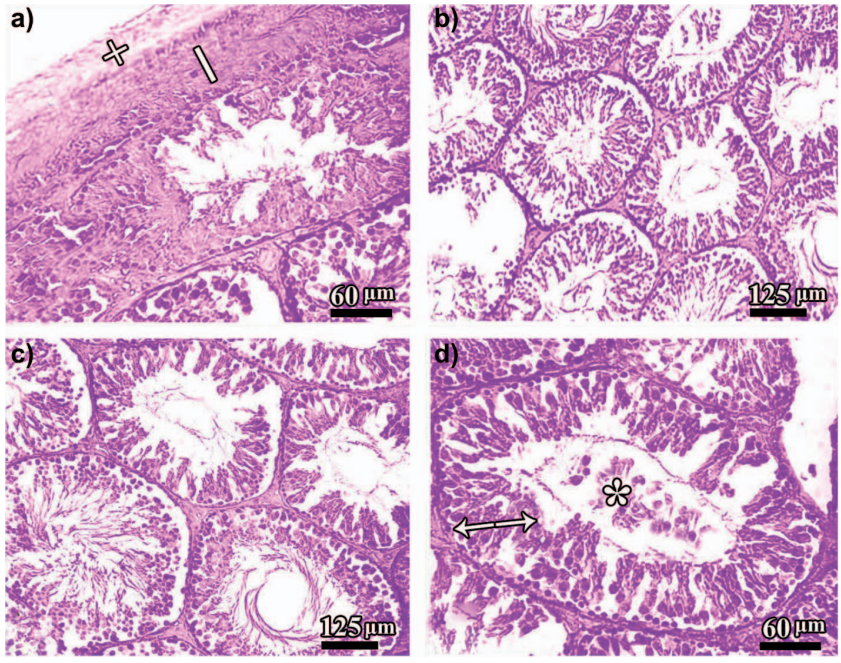

Fig. 4. Experimental group images (a – d). (+) thinned tunica vaginalis; (–) thickened tunica albuginea; double-headed arrow, thinned seminiferous epithelium; asterisk, lumen of seminiferous tubule with decreased diameter.

Statistical analysis

Statistical analysis was performed using Mann Whitney-U statistical test in the SPSS version15.0 packet program) software. Values for $p \leq 0.05$ were considered statistically significant.

Results

Histology

Significant damage to the spermatogenic cells and seminiferous tubules was observed in male rats exposed to Hg^0. Spermatogenic cells had pycnotic nuclei and eosinophilic cytoplasm, and loss of spermatogenic cells was observed in many seminiferous tubules. We observed seminiferous tubule atrophy and a thickened tunica albuginea (Fig. 4). Also, we detected late spermatids in the tubule lumens and thinning of the tunica vaginalis in testis sections of the Hg^0 exposed group. No histological abnormalities were observed in the testes of the control group (Fig. 3).

Stereology

We found significant decreases in the total volume of testes, and diameter and total volume of seminiferous tubules in the Hg^0 exposed group compared to the control group ($p < 0.05$) (Table 1). We found also that the numbers of Sertoli cells, spermatogonia, spermatocytes and spermatids were significantly reduced in the Hg^0 exposed group compared to the control group ($p < 0.05$) (Table 1).

Table 1. Stereological results of the study.

Estimation	Control group	Experimental group
Testicular volume (cm^3)	1.54 ± 0.077*	1.16 ± 0.0058
Seminiferous tubule diameter (µm)	168 ± 8.4*	159 ± 7.25
Volume of seminiferous tubules (cm^3)	1.03 ± 0.0515*	0.87 ± 0.0435
Mean number of Sertoli cells (cell/cm^3)	$7.4 \times 10^6 \pm 370000$*	$6.5 \times 10^6 \pm 325000$
Mean number of spermatogonia	$28.46 \times 10^6 \pm 1420000$*	$24.89 \times 10^6 \pm 1245000$
Mean number of spermatocytes (cell/cm^3)	$158.87 \times 10^6 \pm 795000$*	$145.25 \times 10^6 \pm 727000$
Mean number of spermatids (cell/cm^3)	$475.92 \times 10^6 \pm 2380000$*	$349.18 \times 10^6 \pm 17460000$

*Difference between control and experimental groups, $p = 0.05$.

Discussion

Hg is a pollutant that is toxic to animals (Risher et al. 2003). Elimination of Hg from the body generally is slow (Sallsten et al. 1994). Approximately 80% of inhaled Hg is absorbed by the lung; it is transported through blood and accumulates in the testis (Khayat and Dencker 1983) and semen (Leung et al. 2001). Occupational exposure to Hg^0 disrupts DNA repair (Cebulska-Wasilewska et al. 2005). Administration of Hg causes testicular degeneration and cellular deformation in the seminiferous tubules and a significant reduction in testicular weight (Chowdhury and Arora 1982). Orisakwe et al. (2001) reported that low doses of Hg reduced the number of sperm in the epididymis and increased testicular degeneration in mice. El-Desoky et al. (2013) reported that Hg damaged seminiferous tubules by decreasing the activity of testicular antioxidants, which increased oxidative stress. Exposure to Hg also caused deterioration of membrane integrity and DNA breaks in bull spermatozoa (Arabi and Heydarnejad 2007).

We found that the reduced numbers of spermatogonia, spermatocytes, spermatids and Sertoli cells in the animals exposed to Hg^0 compared to the control group could be due to apoptosis or ischemic necrosis caused by oxidative stress. The decreased volume of seminiferous tubules in the Hg^0 group likely resulted from seminiferous tubule atrophy. Decreased interstitial tissue and total volume of seminiferous tubules and thinning of the tunica vaginalis likely caused reduction of the total volume of the testis.

We demonstrated that Hg^0 exposure exerts toxic effects on the male reproductive system. Further research is required to determine the effects of Hg^0 on other parts of the reproductive system.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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