The outcome of potassium dichromate exposure on histological structure of male rat sexual organs in suckling period

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Abstract

The aim of this study was the evaluation of integrity biomarkers of reproductive toxicity: histoarchitecture of genital organs (testis and epididymis) and sexual accessory glands (prostate, seminal vesicles and bulbo-urethral glands) at sexual maturity after exposure to potassium dichromate (Cr VI) in suckling period. Consequent to the exposure of male rat pups to hexavalent chromium during suckling period structural changes appeared in genital organs and sexual accessory glands, such as: basal membrane and epithelial disintegration, interstitial edema, wavy basal membrane due to a reduction in tubule diameter; Leydig cell necrosis; epithelial smoothing, epithelial necrosis; epithelial cells desquamation and falling in lumen. Exposure to potassium dichromate (Cr VI) during suckling period produced severe congestive and degenerative lesions in genital organs and sexual accessory glands.

Keywords: chromium VI, histology, male, rat

Introduction

Many heavy metals are known to affect testicular structure, spermatogenesis and steroidogenesis in man and animals, leading to infertility (Aruldas *et al.*, 2004).

Chromium is found in many compounds of the Earth's crust (Stoecker, 2004). It exists in several oxidation states, but Cr III and Cr VI species are the most stable and common forms (O'Brien *et al.*, 2003).

Chromium III is essential for human and animal diet, it is poor soluble, with little or no toxicity. In its hexavalent form chromium is 100 to 1000 times more toxic than the most trivalent compounds (Stoecker, 2004).

Effluents from workplaces and industries contaminate the environment affecting man and animals living in those areas (Aruldas *et al.*,

2004). The main sources of hexavalent chromium are electroplating process, stainless steel welding and vulcanizing industries, tanneries (ATSDR, 2012).

The aim of this study was the evaluation of Cr VI exposure impact on some male reproductive biomarkers. The objective was evaluation of chromium level and histoarchitecture of genital organs (testis and epididymis) and sexual accessory glands (prostate, seminal vesicles and bulbo-urethral glands).

Materials and methods

The study has been carried out, initially, on eight white Wistar female rats, which were obtained from authorized Biobase of University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania. All rats were housed in standard

polycarbonate cages in standard laboratory environmental conditions: temperature $22\pm 2^{\circ}\text{C}$, humidity $55\pm 10\%$, light/dark cycle 12:12h. The animals were fed on standardized normal diet and water *ad libitum*. All the animals were handled in accordance with Directive 2010/63/EU and guideline of National Research Council. The Ethical Committee of Banat University of Agricultural Sciences and Veterinary Medicine Timișoara approved the experimental protocol.

Female rats, divided in four groups: three experimental and one control, were exposed via drinking water to potassium dichromate after delivery as follows: E_1 - 25 ppm Cr VI (LOAEL – EPA,

1998); E_2 - 50 ppm Cr VI; E_3 - 75 ppm Cr VI, control received tap water without chromium content. During mating and gestation, females received only tap water. The pups from each experimental group were exposed via milk and drinking water. After weaning and until sexual maturity pups received only tap water without chromium.

At sexual maturity seven male rats from each group, experimental and control, were sacrificed following protocols and ethical procedures (ketamine and xylazine), and samples were collected. For histological assessment were used samples from genital organs and sexual accessory glands which were fixed in ethanol 80° and

Figure 1. Histological section of testis from E_1 group, Hematoxylin-Eosin stain, 400x
a) basal membrane and epithelial desintegration, b) interstitial edema,
c) wavy basal membrane due to a reduction in tubule diameter

Figure 2. Histological section of testis from E_3 group, Hematoxylin-Eosin stain, 200x
a) basal membrane and epithelium degenerative-necrotic lesions, b) interstitial edema,
c) Leydig cell necrosis

embedded in paraffin wax. Then the embedded samples were cut using microtome Cut 4062 Slee Mainz, Germany in order to obtain histological sections of 5-micron thickness. The slides were stained by Hematoxylin and Eosin method, and then examined on Olympus microscope CX 41 provided with Olympus digital photo camera and image analysis software QuickPHOTO Micro 2.2.

Results and discussions

In all three experimental groups, exposed to hexavalent chromium, histoarchitecture of the genital organs and sexual accessory glands was altered. Namely, the lesions were: in testis interstitial edema, wavy basal membrane due to

a reduction in tubule diameter, basal membrane and epithelium degenerative-necrotic lesions, Leydig cell necrosis (Fig. 1, 2); in epididymis interstitial edema, epithelial necrosis and exfoliation, epithelial smoothing (Fig. 3, 4); in prostate, seminal vesicles and bulbo-urethral glands epithelial desquamation and falling in lumen, epithelial segmental necrosis, flattening of the epithelium, fibrinous exudate (Figures 5, 6, 7).

Permeabilization of the testicular barrier and the accumulation of chromium in the testes could explain the occurred structural alterations (Chandra *et al.*, 2007).

Chromium accumulation in the genital organs following exposure is also possible because

Figure 3. Histological section of epididymis from E₂ group, Hematoxylin-Eosin stain, 400x
←epithelial exfoliation

Figure 4. Histological section of epididymis from E₃ group, Hematoxylin-Eosin stain, 200x
a) epithelial exfoliation, b) flattening of the epithelium, c) epithelial necrosis

Figure 5. Histological section of prostate from E₃ group, Hematoxylin-Eosin stain, 200x
a) epithelial desquamation and falling in lumen, b) epithelial segmental necrosis, c) fibrinous exudate

Figure 6. Histological section of seminal vesicles from E₃ group, Hematoxylin-Eosin stain, 400x
← epithelial cells desquamation and falling in lumen

Figure 7. Histological section of bulbo-urethral glands from E₂ group, Hematoxylin-Eosin stain, 400x
a) flattening of the epithelium, b) epithelial desquamation

they represent the target organs (Arulldhas *et al.*, 2004, 2005). As a result of the accumulation of chromium, congestive-necrotic lesions appear in the genital organs and accessory sexual glands. The responsibility of hexavalent chromium for the occurrence of structural changes in the testicles of adult rats has been demonstrated by some researchers (Chandra *et al.*, 2007, 2010; Li *et al.*, 1999).

Hexavalent chromium is responsible for reactive oxygen species production, which damages the cell membrane by modifying the fluidity and permeability of its lipid component (Dobrestov *et al.*, 1977), altering the structural integrity of the plasmalemma (Gutteridge and Halliwell, 2000), which may underlie histological changes.

In adult monkeys exposed to hexavalent chromium, presence of cell abnormalities in seminiferous tubules were also described (Arulldhas *et al.*, 2005).

Jana *et al.* (2006) observed that testosterone is also responsible for the arrangement of the cells in the epithelium of the seminiferous tubes, which due to exposure to hexavalent chromium is disrupted (Chandra *et al.*, 2010).

The decrease of the number, as well as the degeneration of the Leydig cells following the exposure to hexavalent chromium, is also supported by other researchers (Chandra *et al.*, 2010; Trif *et al.*, 2011; Rankov *et al.*, 2010a, 2010b). The decrease of the diameter, but also the hyperplasia of the Leydig cells was also observed (Arulldhas *et al.*, 2005).

Degenerative lesions in testes with disintegration of spermatocytes, atrophy and necrosis of the seminiferous tubes with decreasing diameter, detachment of germ cells from tubular epithelium, lesions present in individuals from experimental groups, coincide with the reports of other authors (Arulldhas *et al.*, 2005; Chandra *et al.*, 2010; Li *et al.*, 1999; Trif *et al.*, 2011; Rankov *et al.*, 2010a, 2010b; Oliveira *et al.*, 2010).

However, Oliveira *et al.* (2010) did not observe histological changes in the testes and epididymis following administration of 10 mg of potassium chromate / kg body weight.

Affection of the structure of the prostate and seminal vesicles, respectively the appearance of interstitial edema, necrosis and epithelial exfoliation, are scientifically supported (Trif *et al.*, 2011; Rankov *et al.*, 2010a, 2010b).

In the literature studied there were no references to the occurrence of structural changes in the bulb-urethral glands following exposure to hexavalent chromium compounds.

Conclusion

Male rat exposure to potassium dichromate (Cr VI) during the suckling period determined severe structural changes in genital organs and sexual accessory glands (congestive and degenerative lesions): interstitial edema, wavy basal membrane due to a reduction in tubule diameter, basal membrane and epithelium degenerative-necrotic lesions, Leydig cell necrosis (testis); interstitial edema, epithelial necrosis and exfoliation, flattening of the epithelium (epididymis); epithelial desquamation and falling in lumen, epithelial segmental necrosis, flattening of the epithelium, fibrinous exudate (prostate, seminal vesicles and bulbo-urethral glands).

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