REGULAR ARTICLE

CADMIUM AND DIAZINON-INDUCED CHANGES IN THE RAT TESTIS STRUCTURE AFTER A PERORAL ADMINISTRATION IN DRINKING WATER

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ABSTRACT

The present study was carried out to elucidate the effects of heavy metal cadmium (Cd) and a nonselective organophosphorus insecticide diazinon (DZN) administration on the testis structures in adult male rats in subchronic toxicity experiments. A total of 40 Wistar 4 weeks old rats were randomized into 4 groups of 10 animals each and dosed with cadmium (Cd) 30 mg/l (group A), diazinon (DZN) 40 mg/l (group B), and in combination of Cd and DZN (30 and 40 mg/l, respectively, group C) per os in drinking water for 90 days. Testicular histology using a light microscopy and morphometry using PC morphometric software M.I.S. Quick Photo were evaluated. The morphometric data supported histological observations at tubular and interstitial level. Reduced seminiferous epithelium (P<0.001) in Cd group showed desquamation of germ cells, cellular degeneration and necrosis. Increase in epithelial vacuoles and dilated blood vessels relative volume (P<0.001) were observed in all experimental groups. Cd and DZN and their combination exerted changes in the reproductive parameters which could be subsequently negatively related to male fertility. These data provide a novel
insight into the reproductive toxicology of Cd-DZN in male rats. However, results did not indicate synergistic or additional effect of simultaneous administration of both toxicants.

**Keywords:** testis, histology, morphometry, rat, cadmium, diazinon, fertility

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**INTRODUCTION**

Global concerns have been raised in recent years over possible adverse effects that may result from exposure to chemicals that have the potential to interfere with the endocrine system (Courant *et al.*, 2007). While some potential environmental hazards involve significant exposure to only a single compound, the most instances of environmental contamination involve concurrent or sequential exposures to a mixture of compounds that may induce similar or dissimilar effects over exposure periods ranging from short-term to lifetime (U.S. EPA, 2000). The toxicity can be modified by simultaneous or sequential exposure to multiple agents in the environment. For some combined or mixed exposures the health effects may increase more than what would be expected from simply adding the effects of the individual components, therefore there is a concern that several less studied complex exposures may have a large impact on our health as a result of combined or mixed effects (Silins and Högb erg, 2011). Predicting risk from exposure to chemical mixtures is complex, as chemicals in mixtures can interact in terms of both toxicokinetics and toxicodynamics (Meek *et al.*, 2011). Heavy metals could adversely affect the male reproductive system, either by causing hypothalamic-pituitary axis disruption or by directly affecting spermatogenesis, resulting in impair semen quality (Mendiola *et al.*, 2011), environmental cadmium (Cd) exposures may contribute significantly to reduced human male sperm concentration and sperm motility (Benoff *et al.*, 2009). Exposure to cadmium has been reported to induce testicular and epididymal damage (Toman *et al.*, 2008; De Souza Predes *et al.*, 2010) and may contribute to male infertility by reducing sperm quality in both humans and rodents (Benoff, *et al.*, 2009; Xu, 2001, Roychoudhury, 2010). Diazinon (O,O-diethyl O-2-isopropyl-6-methyl pyrimidinyl-4-g-1-phosphorothioate) is a nonselective organophosphorus insecticide primarily used for agricultural purposes and is released to the environment through spraying on a wide variety of agricultural crops and at agricultural sites for pest control (ATSDR, 2008). Diazinon induces several neurological and endocrine alterations in humans and different wildlife species (Kojima *et al.*, 2005; Maxwell and Dutta, 2005; Fattahi *et al.*, 2009).
2009), resulting in reduction in genital weights, reduced sperm motility and viability, and increased sperm morphological abnormalities (Abd El-Aziz et al., 1994). Diazinon may also probably enhance the oxidative stress, altering the antioxidant enzymes which again may have adverse effects on normal cell association affecting the seminiferous tubular architecture (Damodar et al., 2012). It is known that environmental contaminants are responsible for a range of noxious effects on various health aspects but little information is available on their possible combined effect on male reproductive function. Hence the objective of the present study was to assess the histological aspects of testis and seminiferous tubular morphometric and cyto-architectural components on exposure to cadmium and diazinon after a sole or simultaneous intake, as both compounds are regularly found in the animal and human food.

MATERIAL AND METHODS

Experimental design

Young, 4 weeks old male rats of the Wistar strain were randomly divided into four groups of ten animals. The males were housed individually in plastic cages under constant temperature (20-22°C), humidity (55±10%), and 12/12 h cycle of light and darkness with access to food (feed mixture M3, Machal, Brno, Czech Republic) and drinking water ad libitum. All experiments were conducted in accordance with accepted standards of animal care in accredited laboratory (SK PC 50004, SUA Nitra). Rats in the group A were dosed with cadmium (CdCl₂, Reachem, Bratislava, Slovak Republic) at 30 mg/L drinking water for 90 days. Group B was exposed to diazinon (Sigma-Aldrich, Seelze, Germany) at 40 mg/L drinking water for 90 days and rats in the group C were given a mixture of cadmium and diazinon (30 mg/L and 40 mg/L, respectively) in drinking water for 90 days. The fourth group D served as a control and received drinking water with no cadmium or diazinon addition. Animals were anaesthetized with ether and sacrificed 90 days after the start of the experiment.

Testis histology

Testes were removed from the scrotum, freed from adherent tissues and weighed. Left testes were fixed in modified Davidson’s solution (Latendresse et al., 2002) and after processing, tissues were embedded in paraffin, sectioned on a microtome into 5 μm sections and stained with haematoxylin-eosine. Ten sections per testis of each animal were randomly
chosen and evaluated histologically. Testicular sections were studied using light microscope (Nikon Eclipse E600, Kawasaki-Kanagawa, Japan) at magnifications 200x for evidence of degenerative changes.

**Testis morphometry**

Morphometric measurements were based on computerized techniques with PC morphometric software M.I.S. Quick Photo and using light microscope Olympus AX 70 Provis (Olympus, Tokyo, Japan). Absolute volume of testicular structures (μm³), relative volume of testicular structures (%), seminiferous tubule surface area (μm²), and seminiferous tubule diameter (μm) were determined using quantitative morphometric methods (Weibel et al., 1966; Uhrín and Kulíšek, 1980).

**Statistical analysis**

Basic statistical characteristics (mean, standard deviation, variation coefficient) were calculated from the values obtained in each group. Comparisons between the groups were assessed by one-way analysis of variance (ANOVA) and post hoc Scheffe’s test using SAS 9.2 Enterprise Guide 4.3 software (SAS Institute Inc., Cary, NC, USA).

**RESULTS AND DISCUSSION**

Morphometric evaluation of the relative and absolute volume of the testes structures is shown in Table 1 and 2. The changes in the testis structure in cadmium-treated group (A) were more extensive when compared with other groups. Reduced epithelial volume (P<0.001) and significant occurrence of intraepithelial empty spaces (P<0.001) indicating loss of germ cells accompanied with vacuolar degeneration in epithelium and lumen contraction (P<0.05) (Tab 1, 2). Connective tissues were generally unaffected, nevertheless, blood vessels appeared dilated and more congested (P<0.001) (Tab 1, 2; Fig 2). The histological evaluation of rat testes in the peroral cadmium treated group revealed the presence of moderate to severe testicular degeneration and necrosis (Fig 2).
Table 1 Relative volume of the testis structures (%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cadmium</th>
<th>Diazinon</th>
<th>Cadmium + Diazinon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X±SD</td>
<td>X±SD</td>
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<td>X±SD</td>
</tr>
<tr>
<td>Seminiferous epithelium</td>
<td>64.82±2.90</td>
<td>53.67±4.31***</td>
<td>67.73±4.81</td>
<td>65.76±2.89</td>
</tr>
<tr>
<td>Intraepithelial spaces</td>
<td>0.15±0.18</td>
<td>12.79±3.93***</td>
<td>5.09±1.46***</td>
<td>2.96±2.04***</td>
</tr>
<tr>
<td>Tubule lumen</td>
<td>23.58±3.03</td>
<td>19.75±4.14*</td>
<td>14.24±3.51***</td>
<td>18.26±4.45**</td>
</tr>
<tr>
<td>Interstitium</td>
<td>10.93±2.03</td>
<td>11.74±3.06</td>
<td>11.77±1.70</td>
<td>12.01±1.43</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>0.29±0.27</td>
<td>2.05±1.36***</td>
<td>1.17±0.51***</td>
<td>1.01±0.56**</td>
</tr>
</tbody>
</table>

Legend: X – mean, SD – standard deviation, *P<0.05, **P<0.01, ***P<0.001

Our observations were in line with changes observed by De Souza Predes et al. (2010). Authors described a marked reduction of seminiferous tubular diameter after the higher dose of cadmium, along with the conspicuous decrease of the tubular volume density, which means that cadmium caused a significant reduction in the relative seminiferous tubule length (De Souza Predes et al., 2010). In our work, there was no evidence of tubular atrophy, seminiferous tubule diameters and surface did not result in any significant change (Tab 3). Our histomorphometric results are in accordance with previous reports confirming that cadmium-induced testicular necrosis occurs after ischaemia due to rupture of the microvasculature (Toman et al., 2008; Wang et al., 2007; Prozialeck et al., 2008).

In the present work, histological findings in testis from peroral diazinon treated group (B) concluded that most tubules did not corroborate a significant change in size of germinal epithelium and interstitial connective tissue except the evidence of vacuolar spaces (P<0.001) within the epithelium, however some others appeared markedly necrotic with degeneration of epithelial cells and only remnants of the basement membrane and reduction (P<0.001) in the luminal area. The tubules were separated by wide interstitial spaces with focal crowding of Leydig cells and contained many severely thickened, dilated and congested (P<0.001) blood vessels (Tab 1, 2; Fig 3). This finding supports previous research, dilatation of blood capillaries in interstitium, ruptured basal membrane and occurrence of empty spaces in the seminiferous epithelium with detached spermatogonia from basal membrane after 4 weeks of diazinon exposure (El-Mazoudy and Abdou, 2009). The changes observed in the testes included increased interstitial space along with sloughing of germinal cells into the lumen due to inhibition of microtubules formation (Akhtar et al., 2009).
Table 2: Absolute volume of the testis structures (µm$^3$)

<table>
<thead>
<tr>
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<td>X±SD</td>
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<td>X±SD</td>
</tr>
<tr>
<td><strong>Seminiferous epithelium</strong></td>
<td>93.58±12.18</td>
<td>87.04±11.34</td>
<td>88.69±18.15</td>
<td>110.70±13.18**</td>
</tr>
<tr>
<td><strong>Intraepithelial spaces</strong></td>
<td>0.22±0.26</td>
<td>20.46±5.53***</td>
<td>6.46±1.43***</td>
<td>4.88±3.37***</td>
</tr>
<tr>
<td><strong>Tubule lumen</strong></td>
<td>34.04±6.12</td>
<td>31.90±6.73</td>
<td>18.56±5.63***</td>
<td>30.69±8.03</td>
</tr>
<tr>
<td><strong>Interstitial tissue</strong></td>
<td>15.68±3.02</td>
<td>18.98±5.05</td>
<td>15.20±2.54</td>
<td>20.20±3.23**</td>
</tr>
<tr>
<td><strong>Blood vessels</strong></td>
<td>0.43±0.40</td>
<td>3.35±2.21***</td>
<td>1.54±0.78**</td>
<td>1.70±0.93**</td>
</tr>
</tbody>
</table>

Legend: X – mean, SD – standard deviation, **P<0.01, ***P<0.001

No atrophic changes were detected in seminiferous tubules at the light microscope level; the seminiferous tubular diameter as well as cross-sectional surface area did not change significantly (Tab 3). These findings are in agreement with the disruption of germ cells alignment, absent or sparse sperms in lumens as well as reduction in Leydig cells size and number in male mice exposed during early life to organophosphate dimethoate, although reduced seminiferous tubule diameter were detected (Verma and Mohanty, 2009). Diazinon exposure could have a negative impact on the testis structure and reproductive performance of males in association with the changes in testosterone metabolic pattern, altering the activity of the direct hypothalamus–pituitary–gonadal neural pathway or a direct influence of diazinon in testes (Maia et al., 2011; ElMazoudy and Attia, 2012).

Rats receiving cadmium and diazinon perorally (group C) showed both normal and damaged seminiferous tubules in testis. Quantitative morphometric analysis determined significantly increased (P<0.001) occurrence of empty spaces in epithelium associated with significant tubule lumen reduction (P<0.01) as a result of sloughed germ cells detached into the tubular lumen (Tab 1, 2). Nonetheless, the seminiferous tubular diameter as well as total tubular areas did not vary significantly (Tab 3). In some cases the tubules showed severe lesions in the form of diffuse necrosis affecting the germinal layer in the seminiferous tubules and interstitial tissue with congested and dilated (P<0.01) blood vessels (Tab 1, 2; Fig 4). On the contrary, the atrophy of the seminiferous tubules associated with a decrease in the relative volume of seminiferous epithelium of mouse testis after a single dose of diazinon observed Sarabia et al. (2009). Except the normal tubules, other tubules were disorganized, lacking the characteristic basal to luminal maturation of germ cells (Fig 4), confirming that the most
vulnerable site of the testis is the seminiferous epithelium which undergoes degeneration and the germ cells desquamate from the Sertoli cells connections into the tubule lumen creating the empty spaces in the epithelium. For some combined or mixed exposures, the health effects may increase more than what would be expected from simply adding the effects of the individual components, therefore there is a concern that several less studied complex exposures may have a large impact on our health as a result of combined or mixed effects (Silins and Högberg, 2011).

Table 3 Seminiferous tubule morphometry

<table>
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<tr>
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<tr>
<td></td>
<td>X±SD</td>
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</tr>
<tr>
<td><strong>Surface area (µm²)</strong></td>
<td>372.19±69.04</td>
<td>367.87±41.14</td>
<td>364.57±26.17</td>
<td>360.21±32.99</td>
</tr>
<tr>
<td><strong>Diameter (µm)</strong></td>
<td>276.22±26.28</td>
<td>281.67±19.58</td>
<td>273.98±9.90</td>
<td>272.55±12.41</td>
</tr>
</tbody>
</table>

Legend: X – mean, SD – standard deviation

CONCLUSION

Cadmium and diazinon administered in sole doses caused significant changes in the testis structure. Necrosis and reduction in seminiferous epithelium and tubular lumen volumes and increase in the intraepithelial spaces and blood vessels volumes were the main changes in the cadmium exposed group. In the diazinon exposed rats, the increase in intraepithelial spaces and blood vessels volumes as well as decrease in tubule lumen volume were the main significant changes observed. However, the peroral coadministration of these compounds did not pose stronger changes and the effects were comparable to the diazinon exposed group. Cd and DZN exerted toxic impact on the reproductive parameters which could be subsequently negatively related to male fertility. These data provide a novel insight into the reproductive toxicology of Cd-DZN in male rats. However, results did not indicate synergistic or additional effect of simultaneous administration of both toxicants.
Acknowledgments: This work has been supported by the grant from the Cultural and Educational Grant Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic (KEGA) project No. 025UKF-4/2012

Figure 1 Control rat testis (200x, HE)
1 – the tubules containing germinal gells at various stages surrounded by the Sertoli cells are predominant; the germ cells are organized in concentric layers, 2 – tubule lumen with released spermatozoa showing the presence of active spermatogenesis, 3 – interstitial connective tissue with Leydig cells, 4 – intact blood vessel

Figure 2 Rat testis after peroral cadmium administration (200x, HE)
1 – seminiferous epithelium with lack of germ cells in the layers, 2 – delumination of the tubules, 3 – interstitium containing formation of cellular debris, 4 – dilated blood vessels, surrounded by cellular infiltration, Arrows – expanded intercellular spaces between germ cells
REFERENCES


S1-S14.
