

Quantitative Analysis of the Rat Testes after an Acute Cadmium and Diazinon Administration

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Abstract

Changes in the rat testes caused by cadmium and organophosphate insecticide diazinon were evaluated using the morphometry methods. 36 hours after an single intraperitoneal administration of cadmium at a dose of 2 mg/kg b.w., diazinon 20 mg/kg b.w., and cadmium-diazinon 2 and 20 mg/kg, respectively, a significant increase in dilatation of blood vessels ($P<0.05$) after an administration of diazinon, significant increase of intraepithelial spaces volume in testes in cadmium exposed group ($P<0.05$) and highly significant increase in intraepithelial spaces volume in cadmium-diazinon treated animals ($P<0.0001$) were observed. Cadmium and diazinon are toxic for male reproduction and can pose a higher reproductive effects when mixture of both toxicants enters the body.

Keywords: cadmium, diazinon, morphometry, rat, testis

1. Introduction

Diazinon is a thionophosphorus organophosphate pesticide (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) entering the environment from agricultural and household application of the chemical to control pest insects [1]. Except of the neurological effects of diazinon, reproductive effects leading to structural testicular changes in bluegill [2], mice [3], and rats [4] have been described. Pina-Guzmán et al. [3] revealed the high sensitivity of spermatozoa exposed during the late steps of maturation to diazinon. Abd El-Aziz et al. [5] revealed that an oral administration of diazinon to rats results in significantly decreased reproductive tissue weights, increased percentage of dead and morphologically abnormal spermatozoa,

decreased plasma testosterone levels. There is no evidence that long-term exposure to low levels of diazinon causes harmful effects in people. Diazinon has not been shown to affect fertility in humans [1] but can affect both animals and man even after a single exposure [6].

Cadmium (Cd) is a toxic metal which still attracts the attention of researchers and the public because its level in food products often exceeds the maximum allowable limits. The harmful effects of cadmium on living organisms are well described especially in animal gonads [7, 8, 9, 10, 11] and have also associated intoxicant cadmium with recent declining male fertility [12]. Cadmium exposure has also been associated with nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity and genotoxicity, teratogenicity, and endocrine and reproductive effects [13, 14].

To understand the body burdens of environmental contaminants, knowledge on the health risks associated with current patterns of exposure is needed, including increased risk resulting from exposures to multiple chemical [15]. As the

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seminiferous tubules comprise approximately 80% of the testicular mass, the morphological measurements of seminiferous tubules are important in the studies of testis tissues [16].

Aim of this study was to find possible interactions between cadmium and diazinon as the combination of reproductive toxicants because of their possible occurrence in the animal and human food.

2. Materials and methods

Mature, 4 months old male rats of the Wistar strain (weighing approximately 410 g) 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, 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 injected with a single dose (20 mg/kg body wt) of diazinon (Sigma-Aldrich, USA) intraperitoneally in physiological solution, rats of the group B were injected with a single intraperitoneal dose (2 mg/kg body wt) of cadmium in the form of CdCl₂ (Reachem, Slovak Republic) in physiological solution, and rats in the C group were given a mixture of cadmium (2 mg/kg body wt) and diazinon (20 mg/kg body wt) intraperitoneally in physiological solution. The fourth group served as a control and received only physiological solution. Animals were anaesthetized with ether and sacrificed 36 h following an experimental administration. Testis were fixed in modified Davidson's solution [17]. After processing, tissues were embedded in paraffin and cut into 5 µm sections and stained with haematoxylin-eosine for morphometric measurements based on computerized techniques with PC morphometric

software M.I.S. Quick Photo and using light microscope Olympus AX 70 Provis (Japan). 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 using quantitative morphometric methods modified by Uhrín and Kulišek [18]. Comparisons between the groups were assessed by one-way analysis of variance (ANOVA) and post hoc Scheffe test using the Statgraphics Centurion XV software.

3. Results and discussion

Mean testicular weight of rats in diazinon treated group was found to be significantly higher ($P<0.01$) from that of the control (1.45±0.25g) while in group with cadmium and diazinon administration a significant testicular weight reduction occurred ($P<0.05$). The reason of this decrease may result from the reduction of the tubular size and decrease in the volume of seminiferous epithelium with suppressed spermatogenesis [19]. An increase in weight of testes could be result of the oedematous effects of organophosphates [20]. Cadmium injected rats showed no significant ($P>0.05$) difference in testis weight in comparison with control.

The quantitative analysis revealed highly significant decrease ($P<0.001$) in mean seminiferous tubule diameter (µm) in all experimental groups in comparison to the control group. However, the reduction was stronger in Cd group than in DZN and Cd+DZN groups. The decrease in diameter of the seminiferous tubule might be the case of germ cell layers disarrangement and consequently decreases in the number of germinal cells [21]. The mean testicular weights and the diameters of seminiferous tubules are given in Table 1.

Table 1. Testes weight and diameter of seminiferous tubules of the rats

Group	Testicular weight (g)	Seminiferous tubule diameter (µm)
Control	1.45±0.25	244.72±42.15
Cd	1.49±0.32	190.67±28.06***
DZN	1.62±0.10**	214.39±42.52***
Cd+DZN	1.29±0.16 *	214.96±35.65***

The values are expressed as means±standard deviation; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Testes are the most sensitive organs to acute Cd-induced damage with irreversible necrosis and haemorrhagic injury [22, 23]. Testes from rats treated with cadmium chloride at a single dose of 2 mg/kg body wt showed various degree of testicular degeneration. Degeneration and necrosis of germinal cells, irregularity and atrophy of seminiferous tubules were observed in histological structure. These alterations resulted in significant reduction in germinal epithelium volume ($P<0.001$) and decrease in volume of the tubular lumen ($P<0.001$). An increase in intraepithelial empty spaces volume ($P<0.001$) was caused by detached germ cells filling the tubular lumen. A significant increase in volume of the interstitial connective tissue ($P<0.001$) with significant dilatation of blood vessels ($P<0.001$) and massive haemorrhagic necrosis were also detected. The histomorphometric results are in accordance with previous observations [7, 11, 12] confirming that cadmium-induced testicular necrosis occurs after ischaemia due to rupture of the microvasculature [22]. Diazinon exposure at dose of 20 mg/kg body wt resulted in significant changes in the testicular tissue structure of adult male rat. The results of morphometric analysis support significant histopathological disorders in seminiferous tubules resulting in tubular degeneration and inhibition of spermatogenesis. Seminiferous tubules underwent the atrophy, marked reduction in size and volume of the seminiferous epithelium ($P<0.001$) appeared. Sloughing of germ cells from disintegrated epithelium into the lumen caused a significant increase of intraepithelial empty spaces

($P<0.001$) and significant delumination of tubules ($P<0.001$). The extension ($P<0.001$) of interstitium by 18.94 % with dilated blood vessels ($P<0.001$), and presence of oedema were observed. Histomorphometry confirmed previous studies of organophosphates effect on testicular tissue [6, 19, 21]. Limited information on reproductive effects of cadmium and diazinon by the combined administration are provided. In our experiment, an intraperitoneal administration of cadmium and diazinon in the same time significantly decreased ($P<0.001$) the relative volume of germinal epithelium and tubule lumen compared to the control, although changes in testicular structure were less expressive than in group with a single administration of cadmium. An increase in capillary size ($P<0.001$) led to the development of interstitial oedema. Vacuolization of the seminiferous epithelium and volume of interstitial tissue were significantly increased ($P<0.001$) only in the diazinon treated group. It is an interesting finding that cadmium in combination with diazinon was not able to damage the testis as extensively as in a single dose. A supposed synergistic or additional effect of acute coexposure to cadmium and diazinon did not confirm the results. This data provide a novel insight into the toxicology of cadmium and diazinon interactions on male reproduction. The results of the effects of intraperitoneal administration of cadmium, diazinon and cadmium-diazinon coexposure on testicular tissue in male adult rats are given in Table 2.

Table 2. Relative volume of testicular structures (%)

Group	Seminiferous epithelium	Intraepithelial spaces	Tubule lumen	Interstitial tissue	Blood vessels
Control	64.82±7.24	0.38±1.02	23.58±8.91	10.93±4.31	0.29±0.53
Cd	44.05±8.10***	11.22±4.81***	8.99±6.27***	33.23±10.56***	2.52±4.54***
DZN	55.16±8.90***	6.90±5.55***	7.14±8.29***	29.87±9.45***	0.94±1.45***
Cd+DZN	49.29±9.74***	6.76±5.78***	13.28±7.72***	29.41±11.51***	1.27±2.58***

The values are expressed as means±standard deviation; *** $P<0.001$

4. Conclusions

The results of this work have shown that acute intraperitoneal cadmium and diazinon intoxication induces structural changes at tubular and interstitial level in the testes of adult rats. Cadmium and diazinon exposure may lead to the

fertility failure. A synergistic or additional effect of acute coexposure to cadmium and diazinon in male rats was not confirmed. Further studies are necessary to assess the effects of environmental risk factors on both animal and human male reproductive health.

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