Light Microscopy of Male Reproductive Organs after $CdCl_2$ Exposure: Focus on Morphometric Analysis of Testis and Sperm Parameters

Maria de Lourdes Pereira[1], Renata Tavares[2], Fernando Garcia e Costa[3]

Abstract— Cadmium is a widely spread environmental pollutant which exerts various toxic effects in humans and animals. The present study was aimed to explore the effects of a lower dose of Cd on testis and sperm parameters of mice and the possible recovery after one spermatogenic cycle without any treatment. Adult mice grouped controls (groups I and III) were administered with saline, and groups II and IV were subcutaneously injected with 0.5mg/kg/body/weight of $CdCl_2$. Animals were sacrificed after 24h (groups I, and III), and 35 days (groups II and IV), respectively. Testis and epididymis from all groups were processed for light microscopy, morphometric analysis of the seminiferous tubules and sperm parameters. Cadmium dosed groups showed significant differences in the progressive motility and immotile sperm ($\rho < 0.05$), compared with controls ($\rho > 0.05$). Spermatozoa with normal morphology and curled or bent tail showed no significant differences ($\rho > 0.05$). The histological features of epididymis sections from treated groups exhibited lesions and together with the morphometric analysis showed significant differences between the seminiferous tubules of Groups II compared to Group I. Although deleterious effects of Cd were noted the recovery study after 35 days showed a great retrieval of lesions in both reproductive organs (groups II, and IV).

Index Terms— Cadmium chloride, acute exposure, testis, epididymis, sperm parameters, fertility

1 Introduction

Environmental pollutants including heavy metals such as cadmium, lead, and mercury adversely affect spermatogensis causing poor semen quality which obvious impact on fertility of exposed subjects. In fact, pollution of Cd is increasing with the development of industries such as those from electroplating, production of batteries, pigments, and alloys. These chemials may have an extremely toxic effect even at low concentrations. In humans acute and chronic Cd exposure can result in damage to various organs such as the liver, kidney and testis [1-3].

Concerns of Cd exposure on reproductive functions and embryonic development were reported [4]. In this review a synopsis of reproductive functions affected by Cd is presented, as well as related mechanisms of action. Disruption of the blood testis barrier producing hemorrhage and edema were proposed for Cd testicular toxicity [1].

Previous studies from our group described some adverse effects of Cd on mouse sperm including DNA fragmentation [5], and other genotoxic effects even using 1mg $CdCl_2$/kg/bw [6]. Thus, a lower dose (0.5mg/kg/bw) of $CdCl_2$ was used in the present investigation to explore in mice the morphometric analysis of seminiferous tubules, sperm parameters, and possible recovery after 35 days without any treatment. In addition epididymis morphology was also evaluated.

2 Material and Methods

2.1 Animals and Treatment

Male ICR-CD1 8 weeks old mice, weighing 30-37g, were purchased from Harlan, Barcelone (Spain). Twenty animals housed appropriately in a vivarium (temperature (22±2ºC), relative humidity and on a light-dark 12h/12h cycle) had free access to water and pelleted food for rodents. After one week of acclimation period mice were allocated in 4 groups: controls (groups I and III) were administered with physiological saline, and groups II and IV were subcutaneously injected with 0.5mg/kg/body/weight of $CdCl_2$ (Sigma. St. Louis, MO, USA).

Animals were sacrificed after 24h (groups I, and II), and 35 days (groups III and IV), respectively. Body and organs weights were recorded. Animal tests and assays were directed in harmony with international guidelines for ethics in animal research.

2.2 Light Microscopy Studies

Left testis and epididymis from all animals were fixed in Bouin´ s solution, embedded in parafine wax, sectioned, and stained with Hematoxylin and eosin (HE) for light microscopy observation. Morphometric analysis of the seminiferous tubules was conducted measuring the diameter of 50 seminiferous tubules of Cd-administered mice and compared with those of controls, using Snakes deformable models as

- *M. Lourdes Pereira: [1]Department of Biology, University of Aveiro, Campus de Santiago, 3010-193, Aveiro, Portugal and CICECO- Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal. E-mail: mlourdesperera@ua.pt*
- *Renata Tavares: [2]Institute of Biomedicine, iBiMED, Health Sciences Department, University of Aveiro, Aveiro, Portugal;E-mail: renata@ua.pt*
- *Fernando Garcia e Costa: [3]Department of Morphology & Function, CIISA Interdisciplinary Centre of Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal. E-mail:fgcosta@fmv.ulisboa.pt*

described previously [7].

2.3 Sperm Count, viability, and motility

Right cauda epididymis from all animals groups were dissected out in 1ml of modified Tyrode´s medium (MT6) for sperm release. The evaluation of sperm parameters (count, viability, and motility) was conducted in accordance with WHO guidelines [8].

2.4 Statistical Analysis

Statistical significance was accessed using the Student's t test. Differences between groups were considered significant when $p < 0.05$.

3 Results and Discussion

During the experimental work, no mortality or signs of toxicity was noted among all groups nor any macroscopic changes on testis and epididymis.

Group I showed no significant difference in relation to body weight before and after the experiment ($p > 0.05$), but the same did not happen with groups II, III and IV ($p < 0.05$). Both the absolute weights of testis and sperm density and acrosome integrity showed no significant ences between all groups ($p > 0.05$). However, the absolute epididymis weights between groups III and IV showed a statistically significant difference ($p<0.05$).

These groups also showed significant differences in the progressive motility and immotile sperm ($p < 0.05$), compared with controls ($p > 0.05$) (Table 1).

Table 1. Density and motility of spermatozoa from all groups of animals.

	Density	Motility		
Group	Concentration (ml) average±sd	Progressives (%)average±sd	Non Progressives (%)average±sd	Immotile (%) average±sd
I	$4.34x10^5±2.18x10^5$	15.92±12,26	15.25± 2,98	68.03±13,03
II	$6.95x10^5±3.26x10^5$	23.84±7,37	17.51±3,58	57.86±6,55
III	$1.54x10^6±2.98x10^5$	39.02±10,03	10.40±6,27	57.86±6,55
IV	$1.60x10^6±8.72x10^5$	21.88±10,75	10.25±2,31	67.87±10,38

*n=5

Sperm parameters (density, acrosome integrity, and motility) did not show any statistically significant difference among groups. However, a significant decline in sperm progressive and significant increase on gametes properties was noted in group IV, demonstrating the effecys of Cd even after 35 days. Finally, the study of the morphology of sperm indicated that the results obtained for the normal sperm and with the folded or curled tail were not statistically different, which suggests that Cd did not cause any damage to the gametes. In this study the value of density/sperm concentration (Table 1) was not significant difference ($p > 0.05$) between groups I and II, and groups III and IV.

Spermatozoa with normal morphology and curled or bent tail showed no significant differences ($p > 0.05$).

Histological HE sections from testis, and epididymis of Cd-exposed mice and controls are displayed on Fig. 1.

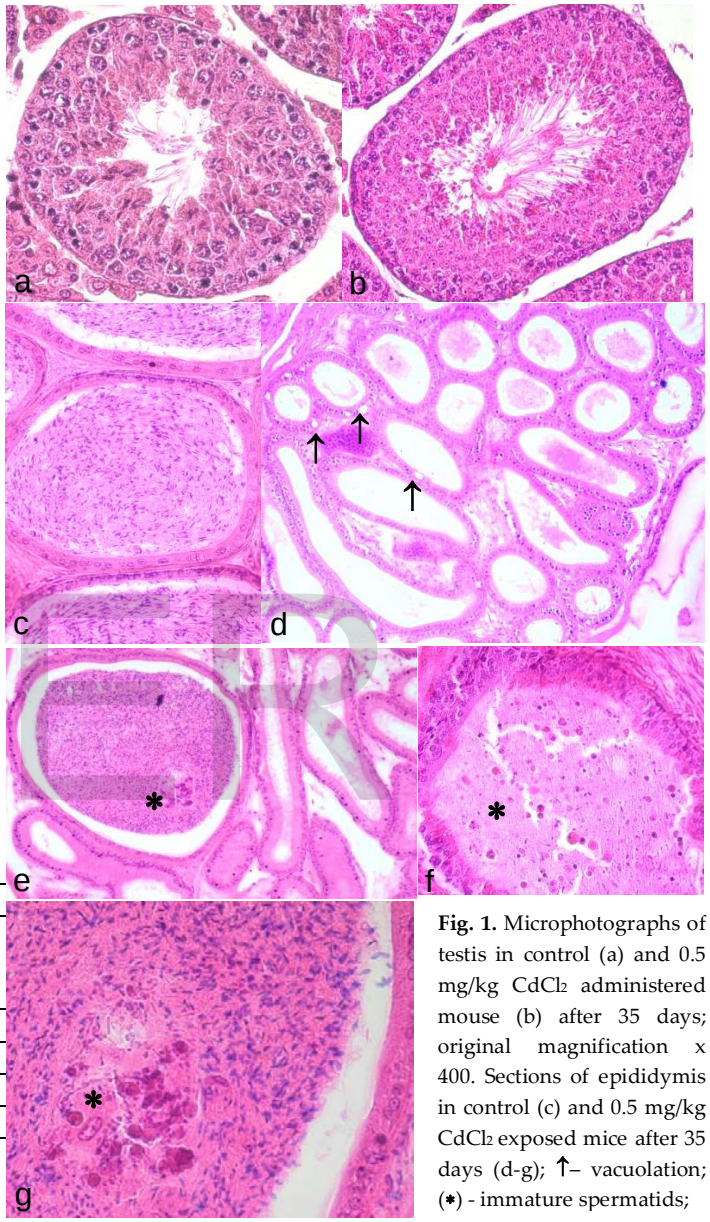

Fig. 1. Microphotographs of testis in control (a) and 0.5 mg/kg $CdCl_2$ administered mouse (b) after 35 days; original magnification x 400. Sections of epididymis in control (c) and 0.5 mg/kg $CdCl_2$ exposed mice after 35 days (d-g); ↑– vacuolation; (*) - immature spermatids; c-f original magnification x400; g - group of immature spermatids (*) x600.

The morphological pattern of testis in control groups, and 0.5 mg/kg $CdCl_2$ administered mouse kept for a period of 35 days without any treatment revealed apparently normal features (Fig. 1a, 1b). However, the morphometric analysis of seminiferous tubules diameter showed significant differences between group II (146.94±14.72), compared to control (156.48±14.68), thus demonstrating the toxicity of Cd. In contrast, group IV showed no significant difference in diameter of the seminiferous

bules (156.98±23.14), compared to the control (164.54±18.69), indicating the recovery of seminiferous tubules diameter after 35 days.

Epididymis of the group II exhibited epithelial vacuolation, and a mass of immature spermatids within the men showing thus the toxicity of Cd after 35 days (Fig. 1d-g) when compared with controls (Fig 1c).

Although deleterious effects induced by Cd were noted the recovery study showed a great retrieval of lesions in both reproductive organs (groups IV).

Deleterious effect on testicular function and biometric parameters of the testes may be important in the assessment of testicular function in rats receiving 15mg/L, 20mg/L and 25mg/L of $CdCl_2$ for 6 weeks [9]. Testicular dysfunction was induced by an oral administration of $CdCl_2$ (5mg/kg/bw every other day) for 30 days [10]. Testicular injury was identified by the reduction in sex organs weight, sperm counts and their motility as well as abnormalities in histological and DNA testicular tissue. Mild histopathological changes on testis such as congested blood vessels, enlarged amount of interstitial connective tissue among others were reported at both concentrations of 0.25 or 0.5 mg/Kg/bwt/$CdCl_2$ [11]. Rats dosed with an acute dose of 1 mg kg^{-1} day^{-1} for 3 days a revealed substantial reproductive damage through increased oxidative stress, morphological changes in testis, and decreased sperm quality [12].

Conclusions

In the present study some degenerative changes induced by lower doses of Cd (0.5 mg/kg) were observed on male reproductive organs of mice such as testis and epididymis but the possibility of recovery was evident after 35 days of the withdrawal of exposure. Although damage on fertility was well documented in the literature due to Cd exposure, studies on molecular approaches are being conducted in our laboratories for a better knowledge of the mechanism underlying those events.

Acknowledgment

This research was supported by Project CICECO-Aveiro Institute of Materials (Ref. FCT UID/CTM/50011/2013), sponsored by national funds through the FCT/MEC and co-financed by FEDER under the PT2020 Partnership Agreement.

4 References

[1] E.R. Siu, D.D. Mruk, C.S. Porto, C.Y. Cheng, "Cadmium-induced testicular injury", *Toxicol. and Appl. Pharmacol.*, vol. 238, no. 3, pp. 240-249, 2009, doi: 10.1016/j.taap.2009.01.028

[2] S. Morais, F. Garcia e Costa, M.L. Pereira, "Heavy Metals & Human Health", Environmental Health - Emerging Issues & Practice, J. Oosthuizen, ed., InTech, pp. 227-246, 2012, http://cdn.intechweb.org/pdfs/27687.pdf

[3] S. Morais, F. Garcia e Costa, M.L. Pereira, "Thoughts on Evaluation of Heavy Metals Toxicity", Advances in Chemistry Research. JC. Taylor, ed., Volume 14. New York, Nova Science Publishers, pp. 273-279, 2012, ISBN: 978-1-61942-327-5. https://www.novapublishers.com/catalog/product_info.php?products_id=38929&osCsid=5e9a553a1b1129fed10cc0ad3598a587.

[4] J. Thompson, J. Bannigan, "Cadmium: toxic effects on the reproductive system and the embryo", *Reprod. Toxicol.*, no. 25, pp. 304–315, 2008, doi: 10.1016/j.reprotox.2008.02.001. Epub 2008 Feb 19.

[5] H. Oliveira, M. Spanò, C. Santos, M.L. Pereira, "Adverse effects of cadmium exposure on mouse sperm", *Reprod. Toxicol*, no. 28, pp. 550–555, 2009, doi: 10.1016/j.reprotox.2009.08.001. Epub 2009 Aug 18.

[6] H. Oliveira, T.Lopes, T.Almeida, M.L.Pereira, C. Santos, "Cadmium-induced genetic instability in mice testis", *Hum. Exp. Toxicol.*, vol. 31, no. 12, pp. 1228-1236, 2012, doi: 10.1177/0960327112445937. Epub 2012 Jun 14.

[7] M.A. Guevara, H. Oliverira, M.L. Pereira, F.A. Morgado, "Segmentation and morphometry of histological sections using deformable models: a new tool for evaluating testicular histopathology", *Progress in Pattern Recognition, Speech and Image Analysis*, A. Sanfeliu & J. Ruiz-Shulcloper, Springer Berlin/Heidelberg. Lecture Notes on Comput Sci, no. 2905, pp. 282-290, 2003, doi: https://www.researchgate.net/publication/220843564_Segmentation_and_Morphometry_of_Histological_Sections_Using_Deformable_Models_A_New_Tool_for_Evaluating_Testicular_Histopathology.

[8] World Health Organization, "WHO Laboratory Manual for the Examination and Processing of Human Semen", 5th ed. Geneva, 2010, doi:http://www.who.int/reproductivehealth/publications/infertility/9789241547789/en/

[9] E.I. Ekhoye, E.K. Nwangwa, C.P. Aloamaka, "Changes in some testicular biometric parameters and testicular function in cadmium chloride administered wistar rats", British J. Medicine and Med. Res., vol. 3, no. 4, pp. 2031-2041, 2013, doi: 10.9734/BJMMR/2013/2821

[10] F.M. El-Demerdash, M.I. Yousef, F.S. Kedwany, H.H. Bahadadi, "Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and b-carotene", *Food Chem. Toxicol.*, no. 42, pp. 1563–1571, 2004, doi: 10.1016/j.fct.2004.05.001

[11] A.Elbetieha, N.Tbeileh, H. Darmani, "Assessment of six weeks exposure to cadmium chloride on fertility of adult male mice", *J. Appl. Biol. Sci.* vol. 5, no. 3, pp. 13-19, 2011, doi:

[12] H.Oguzturk, O.Ciftci, M.Aydin, N.Timurkaan, A.Beytur, F.Yilmaz. "Ameliorative effects of curcumin against acute cadmium toxicity on male reproductive system in rats", *Andrologia*, no. 44, pp. 243–249, 2012, doi: 10.1111/j.1439-0272.2012.01273.x. Epub 2012 Jan 18.