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Original Article

Effects of Dioxin on DNA Damage in the Testes of Adult Male Mice

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ABSTRACT

Background: Given the importance of environmental factors and the impact of environmental pollutants, such as dioxins, on organ systems, especially the reproductive system, it is necessary to study these issues and the mechanisms of their effects.

Objectives: This study aimed to investigate the effects of different doses of dioxins on DNA damage in the testes of adult male mice.

Methods: In this experimental study, 32 male Naval Medical Research Institute (NMRI) rats were randomly divided into four groups: The control group received normal saline, and the dioxin groups were treated with different doses (0.1, 0.5, and 1 µg/kg) for two weeks. Apoptosis in the testes was then examined using a TUNEL assay kit.

Results: The mean number of TUNEL-positive spermatogonia cells was 5.91±5.28 in the dioxin group 1, 7.20±10.03 in the dioxin group 2, and 8.73±4.63 in the dioxin group 3, which was higher than that in the control group (0.16±0.40; P=0.073, P=0.034, and P=0.007, respectively).

The mean number of TUNEL-positive spermatocyte cells was 5.16±1.99 in the dioxin group 1, 2.50±4.62 in the dioxin group 2, and 3.33±2.94 in the dioxin group 3, which was higher than that in the control group (P=0.034, P=0.14, and P=0.037, respectively).

The mean number of TUNEL-positive spermatocyte cells in the dioxin group 1 was significantly higher than that in the dioxin group 2 (2.50±4.62, P=0.047).

The average number of TUNEL-positive spermatid cells was 11.58±6.90 in the dioxin group 1, 11.10±12.19 in the dioxin group 2, and 10.20±7.32 in the 3-dioxin group, which was higher than that in the control group (0.16±0.40; P=0.008, P=0.014, and P=0.015, respectively).

Conclusion: The results of the present study showed that dioxin caused dose-dependent apoptosis in the testes.

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Introduction

Dioxins are among the most toxic environmental pollutants with a high structural strength that may be comparable to dichloro-diphenyl-trichloroethane (DDT). These lipophilic compounds belong to a group of halogenated aromatic hydrocarbons, of which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic and known to be a carcinogen, most of which is released in insecticides and energy production sites, and has four chlorine atoms attached [1, 2].

Dioxins are produced in a wide range of industrial processes, such as metal smelting, production of polyvinyl chloride (PVC) plastics, bleaching of paper pulp with chlorine, production of some insecticides and pesticides, and incineration of waste and coal and petroleum products. Because dioxins are commonly found in the environment, many people have been exposed to them.

However, due to the potentially toxic effects of these compounds, efforts are required to reduce this pollution as much as possible. Dioxins are very durable and stable in the environment, and their half-life is between a few months and a few years. They have low stability in water and low volatility (i.e. they remain in soils and sediments that act as environmental reservoirs, and dioxins can be released from these reservoirs for many years) [3]. Dioxins enter the body of ruminants through ingestion of the soil and plants. Then, humans use the meat and products of these animals. Dioxins cause complications, such as fertility problems, endocrine disorders, immune system damage, and changes in the functioning of the nervous system and endocrine glands [3, 4].

In 2017, Pilsner et al. reported that dioxins reduce sperm parameters and degenerative changes during spermatogenesis. However, it was concluded that the mechanism of action of dioxins is not yet known [5]. Other studies in 2013 reported that dioxins lead to an increase in p450 expression, disrupted endocrine and gametogenesis hormones, and an increase in oxidative stress in Japanese mice in areas with high levels of dioxins [6]. Mohammadi et al. showed that dioxins increase oxidative stress and decrease sperm parameters [7]. In 2009, Jin et al. reported that dioxin gavage 1µg/kg for four days increased the expression of mothers against decapentaplegic homolog 4 (SMAD4) and transforming growth factor beta (TGF-β) and decreased the expression of catalase antioxidant enzymes and glutathione peroxidase [8]. In 2018, Pilsner et al. reported that there was a direct relationship between the serum dioxin concentration of preterm adolescents and the maturation of sperm DNA after puberty [9].

Choi et al. injected 50 mg/kg of dioxins into 40 male rats for four weeks. Dioxin injection reduced testicular weight, sperm and Sertoli cell counts, nephrotic toxin size, and testosterone levels [10].

In one study, the effects of oral administration of 0.75–0.37 and 1.5 mg/kg dioxins were investigated for ten days using the flow cytometry method, which showed an increase in sperm cells with an abnormal shape and a significant increase in apoptosis in dioxin-receiving groups compared to the control group.

Given the importance of environmental factors and the impact of environmental pollutants on various organ systems in the body, especially the reproductive system, it is necessary to study these issues and their mechanisms of effects. In our search, we observed very few studies on the effect of environmental factors especially dioxin on apoptosis in the testes. Therefore, this study was conducted to investigate the effects of different doses of dioxins on DNA damage in the testes of adult male mice aged 2–3 months.

Materials and Methods:

In this experimental study, after the approval of the ethics committee (Code: IR.MUMS.fm.REC.1396.465), 32 NMRI male rats were randomly divided into four groups: the control, 1-dioxin, 2-dioxin, and 3-dioxin groups. The solvent control group received a placebo normal saline and dimethyl sulfoxide (DMSO) and the 1-dioxin, 2-dioxin, and 3-dioxin groups received intraperitoneal 0.1, 0.5, and 1 µg/kg dioxins, respectively, for two weeks. Dioxins were purchased from Sigma (Germany). Apoptosis in the testes was then examined using a TUNEL assay kit.

Investigation of apoptosis with tunnel kit

The mice were first anesthetized with chloroform and after opening the abdominal cavity of their testicle, it was placed in a fixative. Then, the dewatering and clarification steps were performed by passing alcohol and xylene, respectively. The tissues were then molded and cut into 5-micron-thick sections using a microtome, and the cells were stained by Roche's tunnel kit (Merck company, Germany). In short, the paraffin sections were dehydrated. After rinsing in hydrogen peroxide and rinsing, they were incubated with protein kinase k. They were then rinsed and mixed with terminal deoxynucleotidyl

transferase, incubated, and then rinsed and after that incubated and washed with converter-POD. Finally, the samples were incubated with DAB and after washing with Haematoxylin and after dewatering and clarification, they were glued [11, 12]. Positive apoptotic cells were visible under a dark brown microscope and randomly counted in five fields.

Statistical analysis: Finally, the data were entered into SPSS software, version 22 and analyzed by ANOVA and LSD tracking test.

Results

Testicular apoptosis results

Our previous results showed tail deformity as well as a reduction in sperm count and motility [7]. The mean number of TUNEL-positive spermatid cells in the dioxin group was 5.91±5.28 (P=0.073), in the dioxin group was 27.20±10.03 (P=0.034), and in the dioxin group 3 was 8.73±4.63 (P=0.007), which was significantly higher than the control group (0.16±0.40) (Figure 1). The average number of TUNEL-positive spermatid cells in the dioxin group 1 was 5.16±1.99 (P=0.034), in the dioxin group 2 was 2.50±4.62 (P=0.14), and in the dioxin group 3 was 3.33±2.94 (P=0.037), which showed a significant increase compared to the control group (P=0.001). The mean number of TUNEL-positive spermatid cells in the dioxin group 1 increased significantly by 5.16±1.99 compared to the dioxin group 2 (2.50±4.62) (P=0.047). The average number of TUNEL-positive spermatid cells in the dioxin group was 11.58±6.90 (P=0.008), in the dioxin group 2 was 11.10±12.19 (P=0.014), and in the 3-dioxin group was 10.20±7.32 (P=0.015), which showed a significant increase compared to the control group (0.16±0.40). At all three doses, the highest number of TUNEL-positive cells was related to spermatids and the lowest was related to spermatocytes (Figure 2).

Discussion

The results of the present study showed that dioxin dose-dependently caused a significant increase in testicular apoptotic cells. At all three doses, most of the TUNEL-positive cells were related to spermatids and the least to spermatocytes.

In 2017, Pilsner et al. reported that dioxin reduced sperm parameters and degenerative changes during spermatogenesis. However, it was concluded that the mechanism of action of dioxin is not yet known [5]. Other

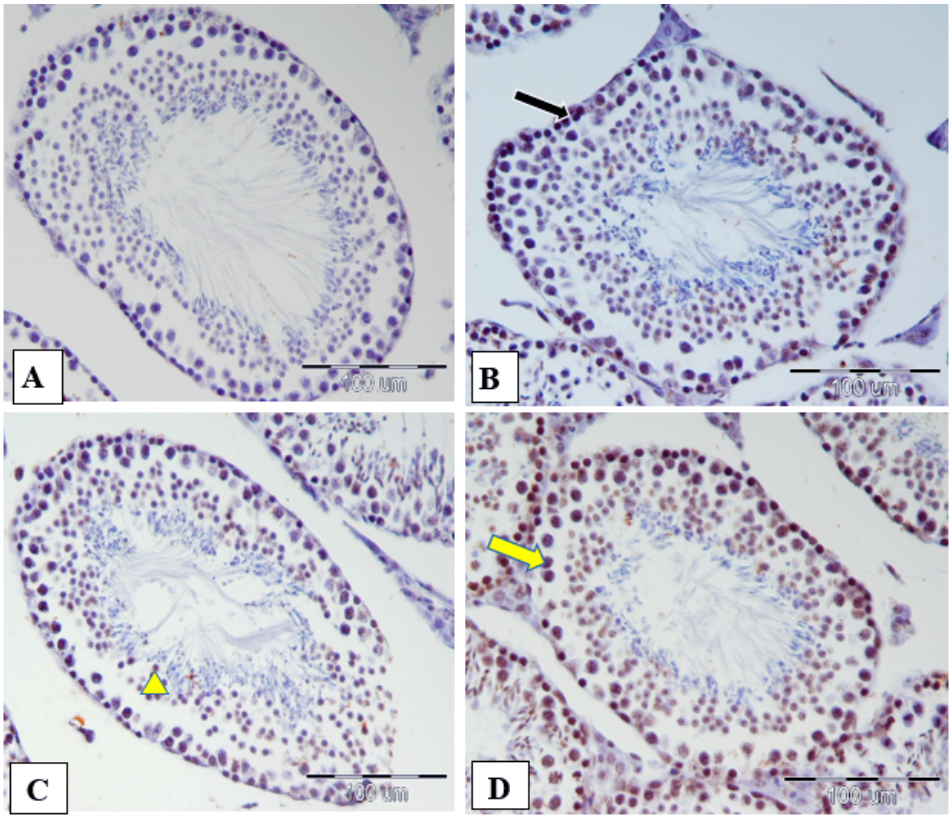

Figure 1. Testicular tissue photomicrograph of different groups using tunnel staining; magnification×400

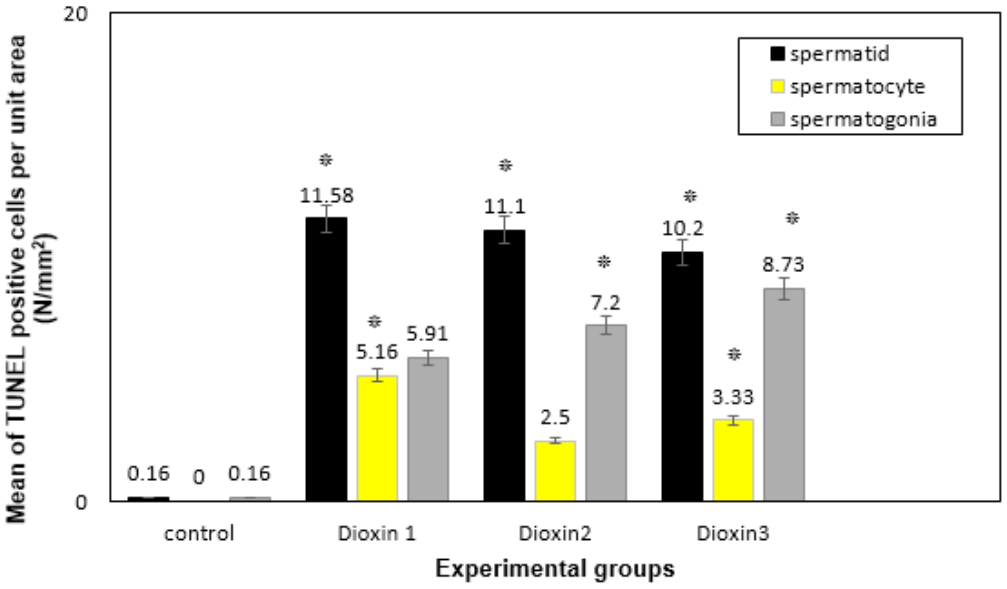

Figure 2. Average number of TUNEL-positive cells per unit area (N/mm²)

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* P<0.05 significant difference between the TUNEL-positive cells and the control group. The mean number of TUNEL-positive spermatogonia cells was significantly higher in the dioxin 1 and 2 groups than in the control group. The mean number of TUNEL-positive spermatocyte cells was significantly higher in the dioxin 1 and 3 groups than in the control group. The mean number of TUNEL-positive spermatid cells was significantly higher in the dioxin 1, 2, and 3 groups than in the control group.

studies in 2013 reported that dioxin led to an increase in p450 expression, disrupted endocrine and gametogenesis hormones, and increased oxidative stress in Japanese mice in areas with high dioxin [6]. Mohammadi et al. showed that dioxin increased oxidative stress and decreased sperm parameters [7]. Compatible with the above studies, in the present study, dioxin had a side effect on testicular tissue and caused apoptosis of testicular tissue.

In 2009, Jin et al. reported that administration of 1µg / kg dioxin by gavage for four days increased the expression of SMAD4 and TGF-ß and decreased the expression of the catalase and glutathione peroxidase [8]. In 2018, Pilsner et al. also reported that there was a direct relationship between the serum dioxin concentration of preterm adolescents and the maturation of sperm DNA after puberty [9].

Choi et al. injected 50 mg/kg of dioxin into 40 male rats for four weeks. Dioxin injection reduced testicular weight, sperm and Sertoli cells, nephrotic toxin size, and testosterone levels [10]. The dose and duration of injection in this study were higher than ours and devastating results were reported.

In a study, the effects of oral administration of 0.75 and 1.5 mg/kg dioxin were investigated for ten days. The results showed an increase in sperm with abnormal shape and a significant increase in apoptosis in dioxin-receiving groups compared to the control group. In this study, the flow cytometry method was used to investigate apoptosis [11]. The dose and duration of injection of our study were close to this study, and in our study, testicular apoptosis was observed after dioxin injection. However, in this study, flow cytometry was used to examine the testicular apoptosis, and in our study, we used the tunnel technique. In our research, the Tunnel Apoptosis Kit was used to detect DNA fragmentation, which is a hallmark of late apoptosis.

In this study, it was better to study the genes that affect apoptosis, which was not done due to financial resources, and it is recommended to other researchers. It was also better to expose the mouse to dioxin in the same way as humans, and not to inject dioxin into them. Besides, in this study, it is better to use positive control as a prevention of the apoptosis effect of dioxin.

Conclusion

The results of the present study showed that dioxin dose-dependently caused testicular apoptosis.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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