EFFECTS OF ANTIDOTAL THERAPY ON TESTIS TISSUE IN ORGANOPHOSPHATE POISONING

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ABSTRACT

***Aims**: The study was aimed to clarify whether toxic effects of organophosphorus compounds on ultrastructural changes in the testis tissue are reversible with antidotal treatment or not.*

***Materials and methods**: In this prospective, controlled, electron microscopic animal study, Wistar albino adult male rats were divided into 3 groups. 1st group received only isotonic sodium chloride. 2nd and 3rd groups received lethal dose of [(LD50) = 30 mg / kg] Methamidophos. 3rd group was treated with atropine and pralidoxime after cholinergic signs had evolved.*

***Results**: In testicular tissue samples of rats in 2nd group, we have found structural degenerative changes. The giant lipid droplets, dense mitochondria, agranüler endoplasmic reticulum vacuolization have been observed. The thickness of membrana propria and electron density of Sertoli have been increased. Also findings of cytoplasmic lysis in most of the tubules, arrest in spermatogenic cells, immature release to lumen and apoptotic changes have been detected. In samples of 3rd group, degenerative changes in testicular tissue were similar to the 2nd group, and no significant structural improvement was proved after treatment.*

***Conclusion**: Our study revealed that acute exposure to Methamidophos causes important degenerative changes in all elements of testicular tissue; and those structural degenerative changes are not reversible with pharmacological drug treatment in the acute phase.*

***Key words**: Methamidophos; poisoning; organophosphates; testis; infertility.*

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Introduction

Widely usage of Methamidophos and other organophosphate derivative insecticides for agriculture leads to environment and air pollution, threatens human health. The consumption of food contaminated with Methamidophos, inhalation and skin contact with these compounds or the use in a variety of ways for attempting suicide leads cellular and biochemical toxicity in humans and other organisms[1].

Besides cellular destruction due to toxic effects on various organs such as nervous system[2], skin[3], liver[4]), kidney[5]; organophosphorus compounds are reported to adversely affect embryonic development[6] and have toxic effects on female[7] and male reproductive system[8] of mice in experimental conditions. With the realization of cessation of spermatozoon production due to pneumonia in humans[9], many researchers have turned to study changes on testicles made by a wide variety of different toxic agents. Indeed, it was reported that carbosulfan causes impairment in testicular function, decrease level of follicle stimulating hormone (FSH) that plays a role in spermatogenic development and cause reduction in number of spermatozoon by toxic effects[10]. In addition, especially regarding the results of intense studies focusing on semen parameters, a significant decrease in semen quality and an increase in abnormally shaped sperm have been reported[11,12]. Although there are studies regarding toxic effects of organophosphorus compounds on different organs and tissues and ultrastructural changes in the testis[8,13,14], there are no studies indicating whether these effects are reversible with treatment or not.

This study was aimed to determine structural effects of acute poisoning by Methamidophos, an organophosphate derivative compound, on rat testis and investigate whether these effects are reversible or not with antidotal treatment at electron microscopic level.

Materials and methods

The study was initiated after obtaining permission from Cukurova University Ethics Committee of Experimental Animals. Wistar albino adult male rats with an average weight of 190-250 g were used in this study. Ambient temperature was kept at 22 ± 2 °C. Ventilation was provided with window type aspirator. The windows were painted black and light-dark cycle was adjusted to be 12 hours light-12 hours dark with an automatic controller device (06:00 to 18:00 light, 18:00 to 06:00 dark). The rats were kept in stainless steel wire cages. Sawdust was used for bedding in the cage and they were replaced once a week. Rats were fed with ready pellet feed and tap water was used for water requirements. All rats were provided free water and nutrient intakes.

Totally 50 Wistar albino adult male rats were divided into 3 groups. Rats in 1st group (control group, n=16) received only isotonic sodium chloride. 2nd group (experiment group, n=17) received lethal dose of [(LD50) = 30 mg / kg] Methamidophos and were waited until cholinergic symptoms evolve. 3rd group (treatment group, n=17) was also given the same dose of Methamidophos but the rats were treated after cholinergic signs had evolved. Methamidophos (0,5- dimethyl-fosforamidothioat, average lethal dose (LD50) = 30 mg/kg) was diluted with tap water to 99.1% percent to be 30 mg/kg and was given by gavage using a 20 gauge-feeding needle.

Cholinergic symptoms appeared within 5 minutes in rats in the experiment group. 8 minutes after administration of Methamidophos, all the signs of cholinergic crisis were seen and rats died. The time of interval until emergence of the cholinergic symptoms was 5 minutes in treatment group as experiment group after administration of Methamidophos. However, all symptoms of cholinergic crisis, providing that treated, were recorded for this group (after 8 minutes). Single dose of 40 mg / kg Pralidoxime (2-PAM) was administered to treatment group. Atropine was also given until cholinergic symptoms regressed. Atropine sulfate and 2-PAM were dissolved in saline and were injected intraperitoneally with 23-gauge needle.

Intracardiac blood samples were taken from all the rats in each group in order to check pseudocholinesterase levels and samples were measured by enzymatic colorimetric method. (S-butyrylthiocholine iodide) (Integra COBAS 800 Roche, Germany).

At the end of the third day, the rats were anesthetized with intramuscular administered 75 mg / kg ketamine and 5 mg / kg xylazine. Rats were positioned in supine position on the operating table. After being sacrificed by intracardiac blood collection method, testicles located in the scrotum were removed immediately with the aid of a scalpel and tweezers. The 1 mm3 testis specimens were fixed in 5% glutaraldehyde which was prepared with Millonig phosphate buffer for 4 h at 4 - 8 C0,washed in the same buffer, and then, post-fixed with 1% osmiumtetroxide (OsO4) in sodium phosphate buffer for 2 h at 4 - 8 C0. The tissues were dehydrated in graded series of ethanol, then these specimens were embedded in araldite. Fine sections from selected areas were cut at 500 A˚ thickness and then stained with uranyl acetate and lead citrate for transmission electron microscopic (Jeol JEM 1400).

Results

Group 1

Membrana propria surrounding the tubules with normal electron density was regular in nature. Basal lamina was clear and smooth under the epithelial cells. Inner non-cellular layer, myoid cells at inner cellular layer, outer non-cellular layer and outer cellular layers were normal (Figure 1).

Sertoli cells resting on the basal lamina had a nucleus characterized by significant indentation. The nucleus consisted of one or two nucleoli. Cytoplasm included tubular cristae and cup-shaped mitochondria, a few lipid droplets, extensive rough endoplasmic reticulum, free ribosomes, lysosomes and developed Golgi complex. Close links were observed between spermatogenic cells. The nucleus was oval shaped and consisted peripheral patch shaped nucleolus in Spermatogonium resting on the basal lamina as Sertoli cell (Figure 1).

Spermatocytes containing nuclei characterized by synaptonemal complexes reflecting spermatogenesis process were at normal electron density. Cytoplasm included cristae mitochondria, microtubules and developed Golgi complex. Acrosomal vesicle was settled to a pole of the nucleus in sper-

matids (Figure 1). Spermatogenic cells connected with cytoplasmic bridges were also present in seminiferous tubules.

Pseudocholinesterase level of animals in the control group was 579.4 ± 59.0 of IU / L.

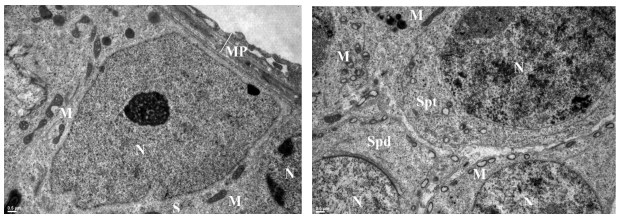

Figure 1: Electron microscopic examination of the 1st group [Normal membrana propria (MP), Sertoli cell with normal electron density (S), Nucleus (N), Spermatogonium (Spg), Mitochondria (M). Normal seminiferous tubules. Spermatocytes (Spt), Spermatids (Spd), Nucleus (N), Mitochondria (M), Bar. 0,5 μm].

Group 2

Electron microscopic examination of testis tissue samples obtained from Methamidophos applied group revealed irregularities of membrana propria in most of the tubules, increase in total thickness, basal lamina becoming irregular, folded and bilaminar, basal lamina making protrusions into tubular epithelial cells, slight collagen fiber increase in inner noncellular layer. Myoid cells with increased electron density included many peripheral micropinocytotic vesicles and cytoplasmic vacuoles (Figure 2).

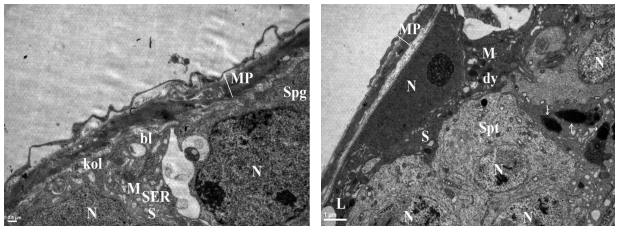

Figure 2: Electron microscopic examination of the 2nd group [Collagen fiber (col) increase and basal lamina (bl) irregularities are observed in irregular membrana propria (MP) with increased total thickness. Irregular gaps (asterisk) are observed between widespread agranüler endoplasmic reticulum (SER) vacuolization exhibiting Sertoli cells (S) and shrunken Spermatogonium (Spg). Irregular nucleus (N), degenerated mitochondria (M), electron-dense structures (ds) and lipid droplets (L) are seen in Sertoli cells with vacuolated cytoplasm and increased electron density. Spermatocytes (Spt) bound with cytoplasmic bridges (arrowheads) and spermatozoa heads (arrows) are observed. Membrana propria (MP), Bar. 0,5 μm].

In some micrographs, Sertoli cells were observed to become electron dens and gaps between them and basal lamina were seen. Nucleus was irregularly contoured and deepening of nucleus indentations were seen. Cytoplasm appeared foamy depending on smooth endoplasmic reticulum vacuolization. Tubular mitochondria consisted dense, swollen and degeneration showing, scattered microtubules in vacuolated cytoplasm. Also electron dense structures and giant lipid droplets had also attracted attention. Abnormal spermatozoa embedded in electron dense Sertoli cell cytoplasm were also observed (Figure 2).

Pseudocholinesterase level of animals in the experiment group was 32.6 ± 17.0 IU.

Group 3

In some of the samples of this group, membrana propria had similarities to control group but some tubules exhibited more degenerative changes. Different degenerative changes were observed in seminiferous tubule cells of tubules with relatively normal membrana propria. Sertoli cells could be identified with indented nucleus with prominent nucleolus. Cytoplasm containing smooth endoplasmic reticulum, lipid droplets of various sizes, dispersed granular endoplasmic reticulum, tubular and cup-shaped mitochondria was similar to the control group (Figure 3). Spermatocytes with normal density wrapped with cytoplasm's of electron dense Sertoli cells had nuclei characterized by synaptonemal complexes reflecting spermatogenesis process. Some degenerated cristae mitochondria were in groups in the cytoplasm and surfaces that come into contact with each other was seen as electron dense (Figure 3).

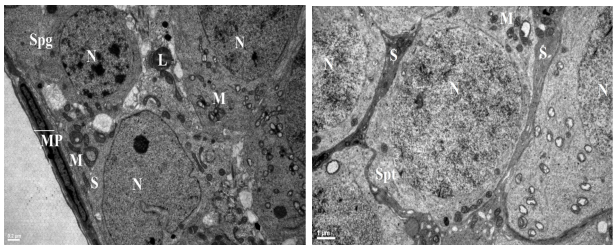

Figure 3: Electron microscopic examination of the 3rd group [Membrana propria (MP), spermatogonia (Spg), nucleus (N), lipid droplets (L), mitochondria (M), spermatocytes (Spt) sertoli cells (S), Bar 0.2 μm].

Pseudocholinesterase levels of animals in the treatment group was 392.5 ± 39.4 IU / L.

Comparison of their own pseudocholinesterase levels between groups was statistically significant ($p = 0.000$).

Discussion

There are many factors that cause infertility in men. One or more deficiencies at sperm development and function are major cause of male infertility. Undescended testis, immotile cilia syndrome, orchitis, torsion, varicocele, chemotherapy, x-ray, medication, heat, or toxins can cause infertility in men. Testicular tissue, where transformation of spermatogonia into spermatozoa occur, must be histologically and biochemically healthy for fertility[15]. In previous studies, organic phosphates derivative compounds such as endosulfan[2], carbendazim[16], methamidophos[17], tamoxifen citrate[18], sypermetrin[19] had been reported to have pathological effects on male reproductive system and cause disorders on semen parameters (sperm count, semen quality). Although there are studies showing effects of Methamidophos, a derivative of organic phosphate, on rat kidney and liver tissue are reversible with atropine and 2-PAM antidotal treatment[4,5], there are no controlled studies showing whether the effects on the testis are reversible or not with treatment.

Methamidophos is widely used in our country to fight agricultural insects. In our study by which it was intended to investigate structural effects of acute poisoning with Methamidophos on rat testis and whether these effects are reversible with treatment, it was found that acute exposure to Methamidophos cause significant degenerative changes in membrana propria, seminiferous tubules, myoid cells, Sertoli cells and all series of spermatogenic cells and mitochondria of cells of testicular tissue. In addition, unlike other studies, in our study, it was found that minimal improvement occurs in degenerative changes with atropine and pralidoxime treatment and these structural degenerative changes are not fully reversible in the acute phase.

The testicles, a part of the male reproductive system, are responsible for production and nutrition of spermatozoa with puberty, and synthesis of testosterone that is the main male sex hormone[20,21]. In each lobule of testis, there are seminiferous tubules where sperm are produced and stroma-containing Leydig cells which are responsible for production of testosterone. Seminiferous tubules are lined with complex, multi-layer epithelium and contain two types of cells: Sertoli cells and spermatogenic cells (spermatogonia, spermatocytes and spermatids). Seminiferous epithelium is surrounded from outside by a specialized fibrous tissue formed by basal lamina, collagen fibers and contractile myoid cells. This fibrous tissue is called membrana propria[22].

In our study, acute exposure to Methamidophos was found to cause degeneration on the structure of the membrana propria. Membrana propria is physiologically very important for spermatogenesis. Membrana propria provides mechanical support to tubule cells, acts as a physiological barrier by transmitting nutrients and metabolites from interstitium into tubule cells and by preventing penetration of chemical and toxic substances that could lead to pathological changes in seminiferous tubules from interstitium into seminiferous tubules.

In our study, swelling and degeneration of mitochondria was observed in spermatocytes and spermatids of rats in experiment and treatment groups. Sperm motility and dysfunction caused by damage to the mitochondria in sperm cells are known to cause infertility[23].

In our study, degeneration was observed in Sertoli cells that are known as supporting cells built-in seminiferous tubules and protect spermatocytes and spermatids from autoimmune reactions with occludens type connections, make up the main structure of blood-testis barrier. Sertoli cells have many functions such as supporting, protecting and nourishing developing spermatogenic cells, releasing peptides that regulate FSH synthesis and secretion, synthesizing androgen-binding protein (ABP), producing anti-Mullerian hormone[20-22].

In studies conducted with other organic phosphorus compounds, these compounds were found to cause degenerative changes that may cause infertility. Malation was found to cause a significant reduction in weight of the testis, epididymis, seminal vesicle, and ventral prostate and decrease in testicular and epididymal sperm density[24]. Chlorpyrifos was also reported to cause a significant reduction in testicular weight in the same way, fall in sperm count, decrease in serum testosterone concentrations, degenerative changes in seminiferous tubules increased in parallel with the increase in dose[25].

Conclusion

As a result, our study revealed that acute exposure to Methamidophos, an organic phosphate insecticide, causes important degenerative changes in all elements of testicular tissue; and those structural degenerative changes are not reversible with pharmacological drug treatment in the acute phase. We concluded that acute intoxication by Methamidophos may cause infertility by severe degeneration of all these structural elements of testicular tissue. New

studies about this topic are required to evaluate the degenerative changes that will occur due to exposure to different doses and durations of organic phosphate insecticides.

References

1) Watson WA, Litovitz TL, Klein-Schwartz W, Rodgers GC Jr, Youniss J, Reid N, Rouse WG, Rembert RS, Borys D. *2003 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System*. Am J Emerg Med 2004; 22: *335-404*.
2) Sherman JD. *Organophosphate Pesticides-Neurological And Respiratory Toxicity*. Toxicol and Ind Health 1995; 11: *33-9*.
3) Liu KH, Kim JH. *In vitro dermal penetration study of carbofuran carbosulfan and furathiocarb*. Arch Toxicol 2003; 77: *225-60*.
4) Satar S, Satar D, Tap Ö, Köseoglu Z, Kaya M. *Ultrastructural changes in rat liver treated with pralidoxime following acute organophosphate poisoning*. The Mount Sinai J Med 2004; 71: *405-10*.
5) Satar S, Satar D, Mete UÖ, Suchard JR, Topal M, Kaya M. *Ultrastructural effects of acute organophoshate poisoning on rat kidney*. Ren Fail 2005; 27: *623-27*.
6) Giri S, Giri A, Sharma GD, Prasad SB. *Mutagenic effects of carbosulfan, a carbamate pesticide*. Mutat Research 2002; 519: *75-82*.
7) Tap Ö, Solmaz S, Polat S, Mete UÖ, Özbilgin MK, Kaya M. *The effect of toluene on the rat ovary: an ultrastructural study*. J Submicrosc Cytol Pathol 1996; 28: *553-58*.
8) Jaiswal A, Perihar VK, Kumar MS, Manjula SD, Krishnanand BR, Shanbhag R, Unnikrishnan MK. 5 – *Aminosalicylic acid reverses endosulfan induced testicular toxicity in male rats*. Gen Toxicol Env Mut 2005; 585: *50-9*.
9) Mills RG. *The pathological changes in the rat testes in epidemic pneumonia*. J Exp Med 1919; 30: *505-29*.
10) El - Zarkouny SA, Ayoub MA, Ishak MAG, El-Nouty FD, Hassan GA, El-Ezz ZRA, Salem MH. *Effect of carbosulfan pesticide and selenium on some semen characteristics and serum testosterone*. Int J Environmental Health Research 1999; 9: *117-24*.
11) Xu LC, Zhan NY, Liu R, Song L, Wang XR. *Joint action of phoxim and fenvalerate on reproduction in male rats*. Asian J Androl 2004; 6: *337-41*.
12) Yang S, Han X, Wei C, Chen J, Yin D. *The toxic effects of pentachlorophenol on rat Sertoli cells in vitro*. Env Toxicol Pharmacol 2005; 20: *182-87*.
13) Francavilla S, Santiemma V, Francavilla F, De Martina C, Santucci R, Fabrini A. *Ultrastructural changes in the seminiferous tubule wall and intertubular blood vessels in human cyptorchidism*. Arc Androl 1979; 2: *21-30*.
14) Farag AT, El-Aswad AF, Shaaban NA. *Assessment of reproductive toxicity of orally administered technical dimethoate in male mice*. Reprod Toxicol 2007; 23: *232-8*.
15) Butt F, Akram N. Se*men analysis parameters. experiences and insight into male infertility at a tertiary care hospital in Punjab*. J Pak Med Assoc 2013; 63: *558-62*.
16) Lim J, Miller MG. *Role of testis exposure levels in the insenitivity of prepubertal rats to carbendazim-induced testicular toxicity*. Fundam Appl Toxicol 1997; 37: *158-67*.
17) Urióstegui AM, Hernández OI, Solís HMD, Martínez AG, Quintanilla VB. *Comparative effect of technical and commercial formulations of methamidophos on sperm quality and dna integrity in mice*. Environ Toxicol 2012 [Epub ahead of print].
18) Rai SP, Vijayalaxmi KK. *Tamoxifen citrate induced sperm shape abnormalities in the in vivo mouse*. Genetic Toxicol Env Mut 2001; 492: *1-6*.
19) Giri S, Giri A, Sharma GD, Prasad SB. *Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice in vivo*. Mutagenesis 2003; 18: *53-8*.
20) Ross MH, Pawlina W. *Histology A Text And Atlas*. Fifth Edition. Philadelphia: Lippincott Williams & Wilkins. 2006.
21) Junqueria LC. *Junqueira's basic histology*. 12th Edition. New York: McGraw-Hill Medical. 2010.
22) Kierszenbaum AL, Tres LL. *Histology and Cell Biology*. Third Edition. Philadelphia: Elsevier Saunders. 2011.
23) Aydos K. *Different Point of View on Spermatozoa Metabolism: Compartmentalization*. J Turkish German Gynecol Assoc 2005; 6: *204-9*.
24) Choudhary N, Goyal R, Joshi SC. *Effect of malathion on reproductive system of male rats*. J Environ Biol 2008; 29: 259-62.
25) Joshi SC, Mathur R, Gulati N. *Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat*. Toxicol Ind Health 2007; 23: *439-44*.

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