New Research about the Effect of Octylphenol (4-tOP) on Histological Structure of Testis in Mice

GABI DUMITRESCU[1], IOAN PET[1], DOREL DRONCA[1]*, MIRELA AHMADI[1]*, LILIANA PETCULESCU CIOCHINA[1], NICOLETA MARIOARA FILIMON[2], ROXANA POPESCU[3]

[1] Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara, 119 Calea Aradului, 300645, Timisoara, Romania

[2] West University of Timisoara, 4 Vasile Parvan Str., 300223, Timisoara, Romania

[3] University of Medicine and Pharmacy Victor Babes, 2 Eftimie Murgu Str., 300041, Timisoara, Romania

The reproductive system of the vertebrates - mainly of the adult males -seems to be vulnerable to the action of endocrine disruptors. The aim of this study was to evaluate the effect of octylphenol (OP) on histological structure of testis in NMRI mice. The mice were divided in three groups (5 mice for each group) such as: control group (C - untreated); vehicle group (V - treated with a mixture of ethanol and corn oil 1:10) and experimental group (E – treated with OP in concentration of 160 mg/kg body weight). The solution of OP was prepared in ethanol, administered by subcutaneous injection in the thigh region (1 ml total volume), for seven consecutive days. For histological examination, after slaughtering, the testes were fixed in 10% neutral formalin, embedded in paraffin, sectioned to a thickness of 5 µm and stained with Mallory's trichrome method. Microscopic analysis reveals in the individuals from the C and V groups, the normal morphology of the seminiferous tubules. In the case of individuals in the E group, treated with OP, in the testicular parenchyma occurs changes consisting in: detachment of the seminiferous tubule epithelium, mild proliferation of germ cells, the presence of apoptotic bodies among the cells of seminal line, diffuse hyperplasia of the Leydig interstitial cells and hypertrophy of the peritubular capillary. Experimental data obtained in this study demonstrated that the treatment with octylphenol induces irreversible changes in the testicular parenchyma with the disruption of spermatogenesis process.

Key words: octylphenol, testis, mice, histology

Vertebrate male reproductive system is very vulnerable to environmental chemicals, particularly to *endocrine disruptors* - which interferes with the action of normal hormones. Starting from this, the purpose of this study was to evaluate the effect of octylphenol (OP) on the histological structure of testicular tissue in mice and evaluate the disruption of spermatogenesis process.

Endocrine disruptors are a heterogeneous group of chemical compounds, natural or synthetic, with hormone mimetic properties [1-3]. These endocrine disruptors were defined by U.S. Environmental Protection Agency (EPA) as exogenous agents that interfere with the synthesis, secretion, transport, binding, action or elimination of hormones [4], reducing the concentration of natural hormones. Also, endocrine disruptors have the ability to bind to estrogenic or androgenic receptors (ER, AR, CAR, AhR, PXR, ERR) and mimic the action of the natural hormone, having agonistic action [5]. Through this action, endocrine disruptors block the receptors and inhibit their action. The effects of endocrine disruptors on health are manifested by alteration of growth function, of development, behavior, production, unbalance the use of storage energy, hemodynamics and blood circulation, but it also affects sexual function and thus, reproduction. These disturbances are more serious as they occur in the early stages of development [6-12]. In addition, endocrine disruptors can induce hermaphroditism [13-15] and may exert direct genetic effects [2, 16-20], which is particularly worrying because it can lead to a number of genetic diseases [21].

In everyday life, humans are exposed to a wide variety of organic and inorganic chemical compounds, encountered in small doses in thousands of products [22-24]. Organic chemicals found more frequently in the human body include dichlorodiphenyltrichloroethane (DDT), polychrolinated biphenyl (PBC), bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs), and a variety of phthalates. Alkylphenol ethoxylates (APEs) are a group of non-ionic surfactants presented in detergents and other cleaning products, hair dyes, pesticides, different lubricants and spermicides.These products (APEs) are microbiologically degraded in several products with estrogenic potential, such as nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), and also 4-tert-octylphenol (4tOP) [25, 26].

The most important representatives of this group are nonylphenol ethoxylate (NPE) and octylphenol ethoxylate (OPE). By metabolic decomposition these substances pass into the environment and lose the side chains of ethylene oxide, forming alkylphenols (4-n-octylphenol and 4-n-nonylphenol). Unlike most exogenous chemicals that usually become less toxic by biodegradation, alkyphenols increase their toxicity during this process of decreasing the length of ethoxylate chain, decreasing also the molecular weight [14].

Nonylphenol (NP) and octylophenol (OP) are ubiquitous pollutants in the environment. Being lipophilic and having high stability, alkylphenols are accumulated and stored predominantly in fat, liver, bile and kidneys of fish and poultry at concentrations of 0.01-0.1 mg/Kg or higher than those found in the environment, and may become available for animal and human consumption [27].

Numerous studies have reported that alkyphenols can influence the correct development and the physiology of the male reproductive system, having as target organs the testicles and epididymis, the reproductive system of

* email: ddronca@animalsci-tm.ro; mirelaahmadi@gmail.com

vertebrates and, particularly the adult males, being mainly vulnerable to the action of these endocrine disruptors.

Alkylphenols are considered responsible for increasing incidence of human reproductive diseases and, consequently, for the decline of reproductive function in human individuals worldwide [28,29]. OP has similarities with natural estrogens, its estrogenic potential being higher than of other alkylphenols. Therefore, the purpose of this study was to evaluate the effect of 4-tert-octylphenol (4-tOP) on the testicular histological structure in mature mice.

Experiment part

In our experiment were used mice - NMRI line, of 2 months of age, having about 30 grams as average weight. The mice provider was Cantacuzino Institute of Bucharest and during the experiment they were housed in specific and proper areas, within university. Animal care conditions have been aligned to the national, international regulations and ethical considerations for animal experiments: the mice were housed in plastic cages on wood shavings, at 21±10ºC, humidity 55±5%, photoperiod 12:12 light-dark. All mice had free access to standard diet (rodents briquettes) and tap water.

The 4-tert-octylphenol (4-tOP noted as OP) was purchased from Sigma-Aldrich (Germany), and other reagents and colorants for the histopathological study were purchased from Merck (Germany). The OP solution in concentration of 160 mg / kg body weight was prepared in ethanol. The OP solution was administered by subcutaneous injection in the thigh region, in a total volume of 1 ml [30], at 24-hour intervals, for a period of 7 consecutive days. The mice were divided into three groups (5 mice / group): control group (C – untreated); vehicle group (V – treated with ethanol and corn oil mixture, 1:10), and experimental group (E – treated with OP 160 mg / kg body weight). At 24 hours, after the last administration, the mice were sacrificed by cervical dislocation. For histopathological examination, after sacrificing the mice the testes were fixed in neutral formalin (10%). Subsequently, they were dehydrated using increasing concentrations of ethyl alcohol (70, 80, 90, 100º), clarified in two benzene baths and embedded in paraffin wax. The sectioning of paraffin blocks was performed with the Leica Rotary Microtome at a thickness of 5 mm; were transferred on microscopic blades; and the histological sections were stained with the Mallory trichromatic method, and examined then with the Olympus CX41 research microscope equipped with a digital camera and image analysis software.

Results and discussions

Microscopic analysis of histological sections of testicles reveals – in the case of individuals in the control group (untreated) and vehicle group (treated with a mixture of ethanol and corn oil - 1:10), the normal morphology of the seminiferous tubules (fig. 1, 2). They are padded with a multilayered polymorphic epithelium, ordered, composed of all types of specific seminal line cells, namely: spermatogonia, spermatocyte I, spermatocyte II, spermatids, and spermatozoa.

Spermatogonies are oval cells, with basophilic cytoplasm, located on the extremely fine basal membrane of the epithelium. Spermatocytes I are disposed on several overlapping rows, large in size, being in different phases of the meiotic division, mainly in prophase. Spermatocytes II and spermatids are also numerous, and at the apical pole of the epithelium there are spermatozoa oriented with their head towards the epithelium and with tail to the lumen of

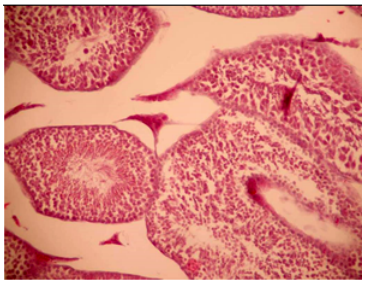

Fig. 1. Testicle-Control group. Seminiferous tubules and small groups of normal-looking endocrine cells [Mallory trichromatic coloring; 100x]

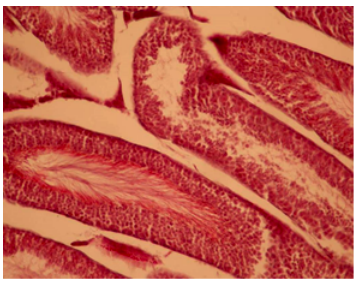

Fig. 2. Testicle - Vehicle group. Seminiferous tubules and small groups of normal-looking endocrine cells [Mallory trichromatic coloring; 100x]

the seminiferous tubules. In the connective tissue between the seminiferous tubules, small groups of polygonal cells are formed, forming the Leydig interstitial gland.

In the case of individuals from experimental group (treated with OP 160 mg/kg body), the microscopic analysis revealed changes in the testicular parenchyma. Thus, we observed seminal tubular epithelium detachment, mild germ cell proliferation, presence of apoptotic bodies between seminal line cells, Leydig interstitial cell diffuse hyperplasia, and peritubular capillary hypertrophy. Thus, in large areas there are detachment signals (fig. 3) of the seminiferous tubules epithelium from the basal membrane, which appears disorganized due to Sertoli cells which decrease in volume, and thus, loss the contacts between them and the seminal line cells. Also, a frequent reported aspect is that of the seminal line cells apoptosis, a process manifested morphologically by the presence of apoptotic cells and apoptotic bodies (fig. 4, 5). As well, to the individuals from experimental group it was observed the thickening of the basal membrane on which the seminiferous epithelium rests, as well as the hypertrophy of the peritubular blood capillaries. Furthermore, in the connective tissue of the peritubular interstitium, the diffuse hyperplasia of interstitial glandular cells occupying all intertubular space is evident, which may represent a compensatory mechanism designed to increase testicular steroidogenesis in response to insufficient testosterone concentration (fig. 5).

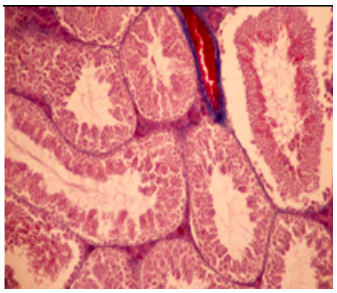

Fig. 3. Testicle-Experimental group (OP). Epithelial detachments, disordered aspect of the epithelium and apoptotic processes [Mallory trichromatic coloring; 200x]

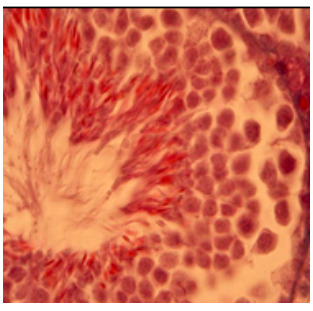

Fig. 4. Testicle-Experimental group (OP). Epithelial detachments, disorganized epithelium and Sertoli cells with low volume [Mallory trichromatic coloring; 1000x]

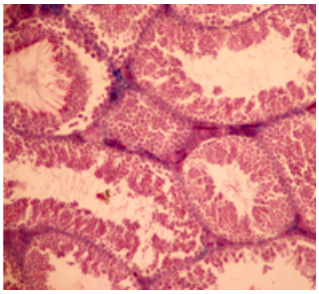

Fig. 5. Testicle-Experimental group (OP). Mild fibrosis in the basal membrane of the seminiferous epithelium and hyperplasia of the Leydig interstitial cells [Mallory trichromatic coloring; 200x]

Similar aspects have been reported following exposure of rats to nonylphenol (NP), in neonatal period, puberty, but also in adults, exposure resulting in histological disorganization of the seminiferous epithelium, reduction of the testes size, of epididymis and seminal vesicles, but also resulting in an increase of cryptorchidism by up to 60% [31]. Changes in the seminiferous epithelium were also reported by Laurenzana and Gong teams [32, 33] occurred as a result of the NP administration in rats. In addition, NP also resulted in a decrease of testosterone concentration.

In the human species, perinatal exposure to bisphenol A (BPA) and estrogens has been demonstrated to influence the fertility by affecting the hemato-testicular barrier [34], as well as by disrupting the junctions between Sertoli cells and seminal line cells [35], interruption of spermatogenesis, but also apoptosis of embryonic cells [36]. Fisher and his collaborators [37] reported that neonatal exposure to high levels of BPA and OP caused major changes in testicular weight. The researcher launched the hypothesis that prolonged exposure to estrogens during fetal and postnatal life could reduce the activity of Sertoli cells, and thus, could reduce the sperm production during adulthood. Also, the exposure to OP causes disruption of Sertoli cell functions as well as Leydig cells and, implicitly, influences masculinization and spermatogenesis functions [6]. Numerous studies have demonstrated that exposure to relevant levels of BPA has adverse effects on testicular function by decreasing luteinizing hormone (LH) secretion and reducing Leydig interstitial cell steroidogenesis [2, 38]. Blake and Boockfor [39] reported that chronic administration of OP in adult male rats interferes with LH secretion, follicle stimulating hormone (FSH) and testosterone. Testosterone is essential to the spermatogenesis process, therefore decreasing LH hormone secretion leads to a decrease spermatogenesis and sperm production.

Development attenuation and function impaired in adult Leydig cells result in decreased testosterone production, as a consequence of reducing the expression of several important steroidogenic factors, including the enzymes StAR, CYP17A1, CYP11A1 and 3HSD. All of these factors are important components of the steroidogenic mechanism and may be targets for the action of endocrine disruptors. Therefore, the inhibitory action of endocrine disruptors on androgenic hormones biosynthesis by Leydig cells can be exerted by attenuating cAMP-PKA signaling, inhibiting the transport of cholesterol by the StAR/PBR complex, and suppressing the expression and/or activity of various steroidogenic enzymes such as 3(3HSD), P450scc, P450c17, 17B HSD [40]. Malfunction of one or more such target proteins by endocrine disruptors can lead to the suppression of androgen production by Leydig cells and the impairment of androgen-dependent physiological processes [2].

A significant decrease in testosterone production was observed in male mice exposed to octylphenol (doses of 2, 20 and 200 mg/kg) in juvenile stage (at the age of 15 days) and in adult stage (at the age of 8 weeks) [39]. Because Sertoli cells proliferation depend on Leydig cell production, the reduction in Sertoli cell proliferation is decreased due to the inhibition of the steroidogenesis process in Leydig cells.

Conclusions

Data obtained by microscopic analysis of histological sections showed that octylophenol (4-tOP) induces changes in both seminiferous and Leydig interstitial cells, reducing steroidogenesis and also testosterone synthesis, altering the spermatogenesis process.

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