Effects of Octylphenol Biodegradation and Toxicity in Biological Systems

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In industrialized countries, many researches have highlighted a correlation between the presence of octylphenol in different plastics or detergents and its estrogenic effect. The metabolism of alkylphenols (4-n-octylphenol and 4-tert-octylphenol) is depending on alkyl chain and at testicular level act as endocrine disruptions. The purpose of our work was to evaluate the octylphenol effect on the testicular morphological changes in mice. Our experiment was performed on adult mice, divided into three experimental groups of 5 individuals each, respectively: control group (C -untreated); the vehicle group (V) treated with ethanol and corn oil mixt (1:10); and experimental group (E - treated with octylphenol in concentrations of 80 mg / kg body weight). Octylphenol is a pollutant that has to be monitoring because our study showed a decrease in male fertility due to morphological alterations as a result of exposure to a series of hormone mimetic molecules. Animals exposed to octylphenol presented morphological changes at the testicular level manifested disorganization of the seminiferous epithelium, hypertrophic and hypereosinophilic spermatocytes, presence of apoptotic bodies, as well as the diffuse hyperplasia of the Leydig interstitial cells, proving that octylphenol negative influences the male fertility.

Keywords: octylphenol, male fertility, endocrine disruptors

Octylphenols are widely used in chemical industry for detergents and different cosmetics, paints, plastics, adhesives, pesticides, rubbers and other commercial products. Also, octylphenols (OPs) are formed in environment by biodegradation of octylphenol ethoxylates (OPEs) very much use in cleaning industry (detergents), but also for paints, coatings products, textile and paper industry. Octylphenol can be found as 4-*n*-octylphenol (4-nOP) or 4-*tert*-octylphenol (4-tOP) (fig. 1).

Octylphenols and their biodegradation forms can enter into the organism – particularly in aquatic organisms - by inhalation, ingestion or dermal contact. These chemicals are under serious concern because they mimic the behavior of sexual hormones, known as *endocrine disruptor*s. Endocrine disruptors are represented by exogenous, natural or synthetic chemical compounds that mimic the action of natural hormones by coupling to specific receptors and blocking their action [1,2]. This leads to disruption of growth and development processes, metabolism, blood circulation and hemodynamics, central nervous system, and sexual function [3-7]. Nowadays, the human population is exposed to various doses of endocrine disruptors such as inhalation of contaminated air; food and contaminated water, daily use detergents, cosmetics, surfaces cleaning, disinfectants, plastics; as well as by widespread use of herbicides and pesticides in agriculture [8-14]. A number of chemicals such as polylchlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDE), bisphenol A (BPA), and a wide range of alkylphenol ethoxylates (APEOs), non-ionic surfactants whose degradation products microbiological (nonylphenol monoethoxylate - NP1EO, nonylphenol diethoxylate - NP2EO, octylphenol - 4-tOP and nonylphenol -NP) potentially estrogenic [15]. The increased solubilization of BPA in containers of beverage and food, led to the global exposure of the human population resulting in early onset of puberty, altered mammary gland development, xenoestrogenic effects manifested also on the male reproductive system [16-19].

Both BPA and 4-tOP have estrogenic activity, although their affinity for estrogen receptors is at least 10,000 times lower than that estradiol [20]. Even if most of the exogenous chemicals reduce their toxicity through biodegradation, it has been studied that-following this biodegradation process-the alkyphenols toxicity increase [21]. Due to their extensive use in a multitude of industries, octylophenol (4-tOP) and nonylphenol (NP) are reported as frequent environmental pollutants. In addition, due to high stability

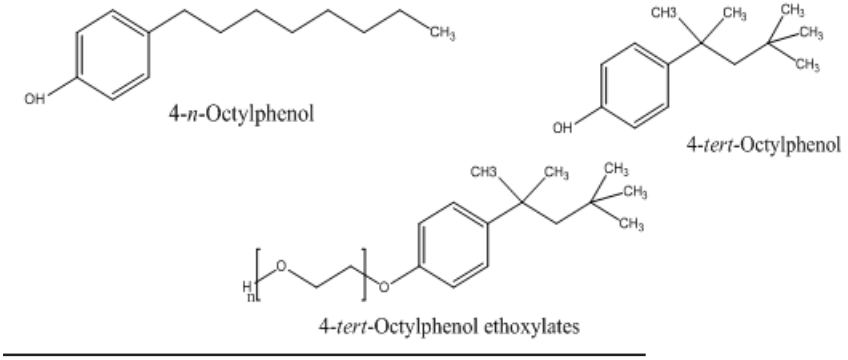

Fig. 1. Chemical forms of octylphenol

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and lipophilic character, alkylphenols can be stored in adipose tissue and different organs (liver, kidneys) rich to higher concentrations compared to those found in the environment. The male reproductive apparatus, and mainly the testicles and the epididymis, represent the main target of alkylphenols action, which are responsible for the morphological and motility abnormalities occurrence, as well as the decrease in the number of spermatozoa [22, 23]. Considering the estrogenic potential of octylphenol, in this paper, our studies have been focused on identifying the main morphological changes induced by different concentrations of this chemical compound in the adult mice.

Experimental part

Biological material

For the experiment we used adult mice - NMRI line, weighing on average 30 g, from the Cantacuzino Institute – Bucharest. During the experiment, the mice were housed in the biobase of BUASVM Timisoara, in very good conditions, respecting specific legislation. The mice were housed in plastic cages, with 12:12 light-dark photoperiod, at 21 ± 1°C and 55 ± 5% humidity, and the standard diet with rodents' briquettes was applied to feed them.

Chemical substances. Reagents and colorants used to perform histological preparations were purchased from Merck (Germany) and 4-tOP was purchased from Sigma-Aldrich (Germany). We prepared the solution of 4-tOP (80 mg/kg body weight) and ethanol solution diluted in corn oil (1:10).

Administration of octylphenol (4-tOP). The 4-tOP was administered subcutaneously in a volume of 1 mL (Kalita, J.C., et al., 1998) for 7 consecutive days at 24 h intervals.

Experimental design

The mice were divided into three groups (5 mice / lot), respectively: control group (CG)- untreated; vehicle group (VG)-treated with ethanol and corn oil (1:10); experimental group (EG) was administered at 80 mg / kg body weight 4-tOP. Mice were sacrificed by cervical dislocation at 24 h after the last administration.

Hystopathologic exam. After male mice sacrifice, the testicles were collected and then fixed in 10% neutral formalin solution, dehydrated in increasing amounts of ethyl alcohol (70, 80, 90, 100%), clarified in two benzene baths, and included in histological paraffin. The paraffin blocks were cut to a size of 5 µm by using a manual rotating microtome and staining was performed by the Mallory trichromatic method. The hystopathologic examination was performed using the Olympus CX41 optical microscope.

Results and discussions

The histopathologic examination performed on the testicles of individuals from control and vehicle group reveal a normal morphological aspect of the seminiferous tubules, captured with an ordered polymorphic epithelium, consisting of seminal line cells and Sertoli cells (figs. 2, 3). Spermatogonies are oval-shaped and are located on the basal membrane of the epithelium. Several rows of spermatozoa I are present in different phases of meiosis, with frequent profanes. A large number of spermatocytes II and spermatids populate the seminiferous tubules, and numerous spermatozoa are seen in their lumen. Among the seminiferous tubes are found small polygonal cells, organized into groups, which constitute the Leydig interstitial gland.

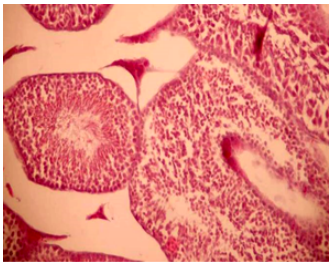

Fig. 2. Testicle - Control group. Seminiferous tubules and normal-looking endocrine cells [Mallory trichromatic coloring, 100x]

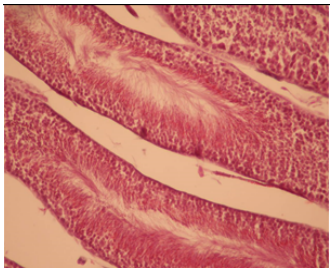

Fig.3. Testicle - Vehicle group. Seminiferous tubules with normal aspect [Mallory trichromatic coloring, 100x]

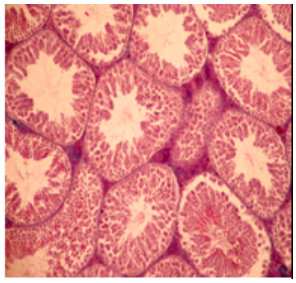

Fig. 4. Testicle -*E.* Epithelium with reduced thickness and intestinal cells hyperplasia [Mallory trichromatic coloring, 200x]

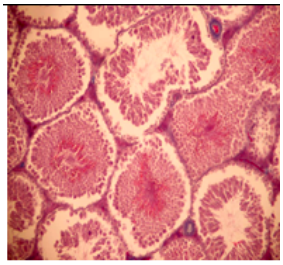

Fig. 5. Testicle-*E.* Detachment of epithelium and cytolysis in tubular lumen [Mallory trichromatic coloring, 200x]

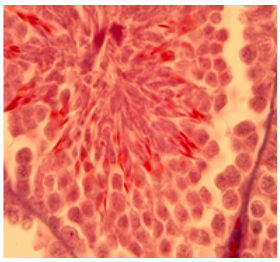

Fig. 6. Testicle- *E.* Detachment of epithelium and the presence of cytolysis in tubular lumen [Mallory trichromatic coloring, 1000x]

In the case of individuals from experimental group (E), to which we administrated 4-tOP in concentration of 80 mg / kg body weight, the epithelium of the seminiferous tubules consists of spermatogonies arranged in a discontinuous layer on the basal membrane, over which some rows of spermatocytes I overlap, mostly in prophase I of meiosis, a small number of cells being detected in telophase (fig. 6). We observed frequently morphological aspect in seminiferous tubers referring to the reduction of the thickness of the seminiferous epithelium (fig. 4). However in a large number of seminiferous tubes, spermatocytes I mainly in the prophase, are placed on 1-2 rows. Instead of this, the spermatocytes II are disordered placed on a few rows, followed by few spermatids and a small number of spermatozoa disposed at the apical pole of the epithelium. In extended areas, the detachment processes of the seminiferous tubes epithelium from the basal membrane are signaled (figs. 4, 5). Also, the epithelium is rarely or disorganized as a result of deletion of seminal line cells and as a result of contact loss between them and Sertoli cells. In the lumen of seminiferous tubules the presence of cell debris and a cytolysis (figs. 5, 6)

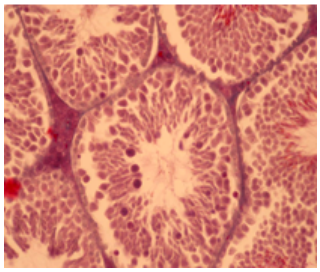

Fig. 7. Testicle - *E.* Epithelial detachments and presence of hypertrophic cells with hypereosinophilic cytoplasm [Mallory trichromatic coloring; 400x]

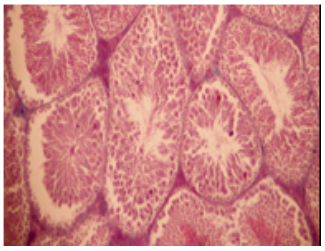

Fig. 8. Testicle -*E.* Epithelial detachments, hypertrophic cells with hypereosinophilic cytoplasm and apoptotic bodies [Mallory trichromatic coloring; 200x]

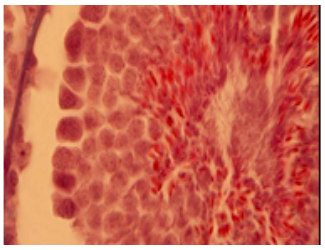

Fig. 9. Testicle -*E.* Epithelial detachments and the presence of apoptotic bodies[Mallory trichromatic coloring; 1000x]

In the seminiferous epithelium, a large number of spermatocytes have a hypertrophic appearance, with hypereosinophilic cytoplasm, which indicates degenerative processes (fig. 7). Similar, but more intense aspects were observed in testicular parenchyma in individuals exposed to 160 mg 4-tOP / kg body weight [24]. In addition, apoptotic cells and a large number of apoptotic bodies (figs.8,9) are reported both in the seminal line cells and in the lumen. This aspect can be correlated with reactive oxygen species (ROS) generation and lipid peroxidation, as a consequence of Ca^{2+} penetration and activation that mediates the NADH complex [25].

Laurenzana and Gong reported a series of morphological changes of seminiferous tubule epithelium, as well as decreased testosterone concentration in rats treated with nonylphenol [26, 27]. Studies on human subjects have also shown that exposure to endocrine disruptors like BPA and estrogen disrupters, leads to damage of Sertoli cells, with losses of contact between them and seminal line cells, disruption of the hemato-testicular barriers and spermatogenesis function, and consequently, affecting the fertility [28, 29]. Sertoli cells are considered *key* elements for the process of spermatogenesis, being of great importance both for the formation of seminiferous tubes and for the functionality of Leydig interstitial cells [30]. In addition, our histopathological examination in the case of individuals from experimental group treated with 80 mg 4-tOP / kg body weight signifies a diffuse hyperplasia process at the Leydig interstitial cell (fig. 9), which is expressed in response to the impairment of the steroidogenesis process. Reduction of testicular steroidogenesis by exposure to xenoestrogens is associated with decreased secretion of luteinizing hormone (LH) and decreased expression of genes for steroidogenesis enzymes in Leydig cells [30]. Studies of Lapointe [31] indicate that endocrine disruptors with estrogenic and antiandrogenic action could negatively influence the spermatogenesis process by abnormally increasing expression of phospholipid-hydroperoxide glutathione peroxidase (PHGPx) in the testicles, an intracellular antioxidant belonging to the selenium-dependent peroxidases. PHGPx interacts directly with cholesterol, with cholesterol esters, and with peroxidised phospholipids, even when are incorporated into lipoprotein biomembranes [32, 33]. Kim [34] indicates that alkylphenolic compounds stimulate the expression of PHGPx mRNA similarly to estradiol. Therefore, it has been demonstrated that both endocrine disruptors with estrogenic and antiandrogenic action amplify the expression of PHGPx mRNA in testicles, suggesting that PHGPx is useful as a biomarker for screening of disrupting effects of exogenous endocrine disruptors at testicular level [36].

Conclusions

Our experimental results obtained by testicular microscopic analysis shown that octylphenol (4-tOP) induces alteration of the seminiferous tubule epithelium and Leydig interstitial cells, suggesting reduction of steroidogenesis, testosterone secretion and, consequently, spermatogenesis disturbance, intensity of morphological changes being concentration dependent.

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