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Histopathological effects of 4-*tert*-octylphenol treatment through the pregnancy period, on the pituitary, adrenal, pancreas, thyroid and parathyroid glands of offspring rats at adulthood

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ABSTRACT

The present study was carried out to investigate the effects of 4-*tert*-octylphenol (OP) exposure at fetal period on adrenal, pituitary, thyroid + parathyroid and pancreas tissues of male and female offsprings. Pregnant rats were treated with OP (100 or 250 mg/(kg day)) in vehicle (corn oil) or vehicle alone daily from day 1 to 20 of pregnancy. After birth, young rats were allowed to growth until adulthood. While there were no differences in data of organ weight between control and treatment groups, in contrast, a decrease of relative organ weights of thyroid + parathyroid and adrenal in high dose treatment group in male rats, otherwise an increase of final body weights was found in 250 mg/(kg day) treatment group in all rats. Also, a lot of histopathological findings were observed in investigated tissues. The results of this study suggest that, the octylphenol which was applied in fetal period causes negative effects on the adrenal, pituitary gland, thyroid + parathyroid and pancreas in rats.

1. Introduction

"Endocrine disruptors" has become increasingly common in the vocabulary of toxicologists over the last 30–50 years the term (Lehtinen and Tana, 2001). These disrupters have been widely produced and distributed basically with the purpose of improving agricultural production. Mainly, herbicides, insecticides, fungicides, polystyrenes, polychlorinated biphenilles (PCB), polychlorinated dibenzodioxines and alkylphenolic compounds can be considered as belonging to these chemicals. These chemicals are usually described as water in soluble or lipophilic. They tend to bioaccumulate in lipids of organisms in the environment. They also have relatively high coefficients for adsorption onto sediments and soils. Alkylphenolic compounds are derived from biodegradation of nonionic surfectants, alkylphenol ethoxylates, which are widely used as detergents in industry. APs such as nonyl or octylphenol are estrogenic in many organisms such as fish, mice, rat, etc. The inappropriate exposure to endocrine disruptors continues to be a significant, as well as controversial, health issue (Colborn et al., 1993; Safe, 1995; Toppari et al., 1996). Most of the endocrine disrupting chemicals (EDCs) which are found in the environment and disrupting the endocrine system have different mechanisms that can influence reproduction of animals and endocrinal control of growth (Neubert, 1997). Endocrine system is a complex system including high homeostasis interactions. Besides, normal endocrinal functions are not stable, and depend on cyclic models, age and developmental phases (Pryor et al., 2000). Exposure to endocrine disrupters in prenatal and postnatal phases can result in long-term impacts (Aydogan and Barlas, 2006). Exposure to estrogenic and anti-androgenic EDC during the developmental phases causes abnormalities in reproductive system, an increase in tumor risks in target organs and functional defects in the reproductive system (Daston et al., 1997).

In recent years, the possibility that manmade estrogenic, antiestrogenic and anti-androgenic endocrine disrupting chemicals can cause strong pathological impacts on human health has been an interesting research study (Yoshida et al., 2001). These chemicals caused significant damages on male reproductive system and it's development (Toppari et al., 1996). And current studies dealing with endocrine disrupting chemicals have revealed a noticeable decrease in the number of sperm counts (Yoshida et al., 2001; Aydogan and Barlas, 2006). In addition to this, there has been a remarkable increase in testicular cancer and congenital abnormality incidents in many countries around the same time period (Carlsen et al., 1992; Adami et al., 1996; Toppari et al., 1996; Paulozzi et al., 1997). And the decline in human male reproductive health (low sperm counts, hypospadias and cryptorchidism, etc.) is caused by prenatal exposure to environmental estrogens (Hossaini et al., 2003). But estrogen affects not only the reproductive system, but also the immune, endocrine and

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central nervous systems. These findings not only coincide with noticeable differences in living conditions, but also the increase in the numbers of industrial and agricultural chemicals including synthetic chemicals. It is well-known that exposure to natural and synthetic estrogens in the neonatal phase causes irreversible hypothalamus–hypophysis–gonadal fractions in both male and female animals (Yoshida et al., 2001). It is shown that some EDCs have low estrogenic activities (Roy et al., 1997) and neonatal exposure to these chemicals is believed to have harmful consequences (Fisher et al., 1999).

p-tert-Octylphenol, which is an anionic substance, is used in detergents in industry and agriculture as an emulsifier, distributor and heater and is formed as a fraction product of alkylphenol ethoxylates (Blackburn and Walddock, 1995). Approximately 500,000 tons are produced annually worldwide. Although estrogenic potential of octylphenol regarding in vitro 17β-estradiol is 100–1000 times weaker, because of its estrogenic effect it is considered as a typical EDC (Blake and Boockfor, 1997). It is reported that overexposure to octylphenol under the skin has caused estrogenic impacts on female rats with no ovaries and rats with a normal cycle on conditions that can change depending on the dose and time (Katsuda et al., 2000; Yoshida et al., 2000). As for adult rats, it is shown that repeated under skin applications with high octylphenol doses caused serious degenerations in male reproductive system with the estrogenic impacts of octylphenol (Blake and Boockfor, 1997; Boockfor and Blake, 1997). Exposure to this compound as neonatal results with irreversible damages on both male and female reproductive organs (Tyl et al., 2000). However, its impact on developing male reproductive and endocrine systems is still unclear, because hormonal changes as well as morphologic changes should also be examined depending on time.

A number of chemicals released into the environment disrupt endocrine homeostasis in humans and animals by interfering with their strictly controlled developmental processes and endocrine system. However, little is known about the effect of octylphenol on the endocrine system. For these reasons we investigated the effects of octylphenol (OP), an estrogenic compound, exposure on the male and female endocrine system during the fetal period.

2. Materials and methods

2.1. Test chemicals

4-*tert*-Octylphenol [4-(1,1,3,3-tetramethylbutyl) phenol] was obtained from Merkolab Chemistry (Ankara, Turkey) with purity of %97 and dissolved in corn oil (vehicle) before use.

2.2. Animals

Twelve-week-old female Wistar albino rats were purchased from the Experimental Animals Production Center, Hacettepe University in Ankara, Turkey. The animals were allowed at least 1-week acclimation interval prior to start the study. Following the acclimatization period, all animals were individually wire-mesh-cages suspended over cage board. The animal room was maintained at a temperature of $22 \pm 2\,^{\circ}C$ and relative humidity 50 ± 5 with a 12-h light/dark cycle (06:00–18:00 h), and given standard rat diet (Korkutelim Feed Factory, Afyon, Turkey) and water were provided *ad libitum*.

2.3. Dose levels

OECD (Organization for Economic Cooperation and Development) recently proposed 28-day repeated toxicity test using adult rats for assessment of toxic effects of environmental chemicals (Yoshida et al., 2000). In our study, we used 21-day repeated octylphenol treatment during pregnancy period. Octylphenol has previously been examined for reproductive disorders in the rats. The doses used and the period of the present study were based on our previous results (Aydogan and Barlas, 2006). In other study (Boockfor and Blake, 1997) adult rats were exposed subcutaneously for 1 or 2 months. OP injected at a dose of 80 mg per animal (corresponding to approximately 400 mg/kg). In another study (Tyl et al., 2000), the adverse effect of octylphenol administrated at dietary concentrations of 0, 0.2, 200 or 2000 ppm were evaluated in two-generation reproduction study in rats. Yamasaki et al. (2003), tested 18 chemicals, they administrated 50, 200 and 600 mg/kg octylphenol. However, 600 mg/kg was reduced to 400 mg/kg dose because of the toxic signs. There are several administrations for testing octylphenol. In our study, we used 100 and 250 mg/kg dose of octylphenol. Doses were chosen to reflect the reported estrogenic potency of the octylphenol in vitro.

2.4. Experimental procedure

The animals were paired for mating in the home cage of the male. Following positive identification of mating or the day when sperm was detected in the vaginal smear was considered to be day 0 of pregnancy. Pregnant rats were removed, distributed on a random basis into control (vehicle) and treatment groups and housed individually. The dams received 100 and 250 mg/(kg day) octylphenol by subcutaneous (s.c.) injection during the gestation period. The injection volume was 0.25 ml/kg body weight in all groups, and the administration volume for each pup was individually adjusted according to the body weight on each day of treatment. Control groups received only corn oil in equal amounts as in experimental groups (vehicle, 0.25 ml/kg). Dams were daily examined for obvious signs of illness. Maternal weight was recorded weekly to pregnancy period. After delivery, all pups were allowed to grow with dam for 1 month. Then, pups were separated from dams. Female and male pups were housed at four per cage and allowed to free access for standard rat diet and tap water *ad libitum*. The pups were allowed to grow up until adulthood (2.5 months of age). Food and water intake was performed daily and body weights for all groups were recorded weekly during the experiment to exclude that the effects of octylphenol were result of a reduction in food and water intake. Then pups were submitted to euthanasia by ether and sacrificed by servical dislocation and then adrenal, hypophysis, pancreas, thyroid and parathyroid of male and female offsprings were removed and investigated morphologically and histopathologically. All experimental procedures and animal use were confirmed as the Approval of Ethics Committee of Hacettepe University.

2.5. Histopathology study

Randomly selected rats from each group were anesthetized under ether and sacrificed by servical dislocation. For histopathological examination, pituitary gland, thyroid, parathyroid (thyroid and parathyroid were removed together), pancreas and adrenal tissues were dissected out and weighed in order to calculate the relative organ weights for each animal. Subsequently, the tissues were fixed in Bouin's solution for 8 h. The tissues were processed in a series of graded ethanol, embedded in parafin. Sections 5 μm thick were obtained and stained with hematoxylin-eosin (for general investigation) and Azan stain (for searching collogen fibril creation and general investigation), to further analysis in a light microscope.

2.6. Statistical analysis

Prior to parametric tests, Kolmogorov–Smirnov tests were used, respectively, to evaluate data for normality and homogeneity. All values presented in the text are mean ± standard error (S.E.). Statistical analyses were performed using a SPSS 13.0 program for Windows. Body and organ weights examined by means of univariate analysis of variance (ANOVA) using a one-way factorial design. Body, absolute, and relative organ weights were examined by Hochberg's GT2-method or Games-Howell, which is based on unequal sample sizes, to detect differences among groups. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Morphological investigation

The maternal findings (pregnancy day, initial and final weights) of pregnant rats of controls and treated subcutaneously 100 and 250 mg/(kg day) octylphenol during the pregnancy period are presented in Table 1. There were no differences in observation data from pregnancy day, initial and final weights of pregnants. But we found that statistically significant decrease of body weight gain in 250 mg/(kg day) octylphenol treatment group than control and 100 mg/(kg day) octylphenol treatment group. Food and water consumption during gestation period was similar in all groups (data not shown).

Data obtained from organ weights (adrenal, pancreas, thyroid + parathyroid) and organ/body weight ratios of dosed female offsprings are presented in Table 2 and male rats are presented in Table 3. In all female rats (Table 2), the final body weight of the 250 mg/(kg day) OP treatment group was increased compared with the control and 100 mg/(kg day) OP treatment groups. The thyroid