

Effects of 4-tert-Octylphenol, during Pregnancy Period, on Infanticidal Behavior in Rats

Gebelik Süresince Uygulanan Oktilfenolün Sıçanlarda İnfantisidal Davranış Üzerindeki Etkileri

Research Article

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ABSTRACT

Infanticide has been observed in numerous animal species, including rats. The present study was carried out to investigate the behavioral effects of 4-tert-Octylphenol (OP) exposure at fetal period on male and female rat 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 day 20 of pregnancy. After birth, infanticidal behavior was observed in 100 mg/kg/day (OP100) and 250 mg/kg/day treatment group (OP250). 47% of live offspring were killed and consumed in OP100 and 53% of live offspring were killed and consumed in OP250. The results of this study suggest that, the octylphenol which was applied in fetal period causes negative effects on the rats.

Key Words

Environmental estrogens; octylphenol; infanticide; rat.

ÖZET

İnfantisit, sıçanların da dahil olduğu bir çok hayvan türünde gözlenmiştir. Bu çalışmada, 4-tert-oktilfenole (OP) gebelik döneminde maruz kalmanın erkek ve dişi yavru sıçanlar üzerindeki davranışsal etkileri araştırılmıştır. Gebe sıçanlar, gebeliğin 1-20. günleri süresince 100 mg/kg/gün (OP100) ve 250 mg/kg/gün (OP250) olmak üzere oktilfenole maruz bırakılmış, kontrol ve yağ kontrol olarak gruplanmıştır. Doğum sonrasında infantisidal davranış OP100 ve OP250 uygulama gruplarında gözlenmiştir. Yavru sıçanlar, OP100 grubunda %47 oranında ve OP250 grubunda %53 oranında yenerek öldürülmüştür. Bu çalışmanın sonuçları, fetal dönemde uygulanan oktilfenolün sıçanlarda olumsuz etkilere neden olduğunu ortaya koymaktadır.

Anahtar Kelimeler

Çevresel östrojenler; oktilfenol, infantisit; sıçan.

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INTRODUCTION

Over the last 30-50 years, many chemicals in the environment have been identified as environmental estrogens [1]. Mainly, phytoestrogens, a number of pesticides and herbicides, some polychlorinated biphenyls (*o,p*-DDT and methoxychlor), some polycyclic aromatic hydrocarbons, and some alkylphenolic compounds (e.g., octylphenol and nonylphenol) can be considered as belonging to these chemicals. The inappropriate exposure to endocrine disruptors continues to be a significant, as well as controversial, health issue [2-4]. Exposure to endocrine disruptors in prenatal and postnatal phases can result in long-term impacts [5,6]. 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 [7]. It is shown that some endocrine disruptors have low estrogenic activities and neonatal exposure to these chemicals is believed to have harmful consequences [8,9].

p-tert-Octylphenol (OP) is an alkylphenol that is an intermediate in the production of alkylphenol ethoxylates. OP is used in detergents in industry and agriculture as an emulsifier, distributor and heater and is formed as a fraction product of alkylphenol ethoxylates [10]. Although estrogenic potential of octylphenol regarding *in vitro* 17 β -estradiol is 100-1000 times weaker, because of its estrogenic effect it is considered as a typical EDC [11]. As for adult rats, it is shown that repeated under skin applications with high octylphenol doses caused serious degenerations. Exposure to this compound as neonatal results with irreversible damages on both male and female reproductive organs [12]. 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.

In rodents, reproductive events induce changes in maternal responsiveness. Immediately upon appearance of the first born, parturient female rodents display a full complement of maternal behaviors, such as pup retrieval, grooming and crouching over the pups. In contrast, virgin female

rodents often avoid contact with pups or attack them [13,14]. In rats, the appearance of maternal behaviors and a decrease in infanticidal behaviors usually occur within 24 or 48 hours before the birth of the offspring. Hormones are an important factor in these changes because mimicking the hormonal changes characteristic of pregnancy induces maternal behaviors in naive female rats [15]. This suggests that, under natural circumstances, physiological changes associated with pregnancy prepare the brain for parental behavior [14].

Infanticide, which means killing young of one's own species, is found in a wide variety of animals, ranging from protozoa and rotifers to birds and mammals, including rats. In rats, the infanticidal behavior may be afforded by the mother. Most infanticide is directed at newborn rats. Newborn pups are far more likely to be killed by adults than 10-12 day old pups [16,17]. Newborn rats appear to taste good to rats. Adult rats who had previously killed neonatal rats readily consumed 18.5 grams in two hours of them, even though the adults' normal food was present. Taste, odor, lack of movement, and lack of hair may all play a role in making a dead neonatal rat an attractive food item [17].

The killing of deformed, sick, and weak pups and the consumption of already-dead ones are different from the killing of healthy offspring. The consumption of an entire litter of pups by the mother may be different from the ingestion of part of a litter followed by good care of the remaining offspring [18]. Most maternal cannibalism occurs in the first 24 hours, and tapers off steeply during the first week [18-20]. However, in rare cases a mother may kill older offspring [21]. Females are more likely to consume the deformed offspring than their healthy siblings, and are more likely to eat already dead young than live ones [20].

Live congenitally malformed offspring were killed and consumed, contrasted to normal live offspring. Regarding stillborn offspring, mothers consumed 63% of the congenitally malformed ones [19]. When the entire litter was alive and normal, there was no cannibalism at all, but most of the litters containing some malformed pups experienced infanticide. Which infants were cannibalized was variable, however: in one case, a female killed a

normal pup and left the malformed one, in another case a female killed one malformed pup but left the others who had the same defect, and in three cases the females consumed the entire litter [19,22].

Helander and Bergh [23] found that newborn rats who are wounded may also be killed. The more wounded the pup, the more likely it was to be killed by the mother. The mothers then proceeded to provide good care to the remaining offspring [18].

The killing of malformed offspring seems adaptive, as it permits females to adjust the final composition of their litters based on environmental and physiological conditions at birth. By removing the pups who are least likely to survive, the mother can give more resources to the remaining pups, increasing their chances of survival, and she can recover some proportion of the resources allocated to the malformed pups through cannibalism [24-26].

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 [1,2,4,27]. However, little is known about the behavioral effects of endocrine disruptors. For these reasons we investigated the effects of octylphenol (OP), an estrogenic compound, exposure on the pregnant rats.

MATERIALS AND METHODS

Test chemicals

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

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 one week acclimation interval prior to study start. Following the acclimation period, all animals were individually wire-mesh-cages suspended over cage board. The animal room was maintained at a temperature of $22\pm 2^{\circ}\text{C}$ 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, Turkey) and water were provided ad libitum.

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 birth, all litters were counted and observed from first minute till infanticide. All experimental procedures and animal use were confirmed as the Approval of Ethics Committee of Hacettepe University.

Statistical analysis

Prior to parametric tests, Kolmogorov-Smirnov tests were used, respectively, to evaluate data for normality and homoscedasticity. All values presented in the text are mean \pm standard error (S.E.). Statistical analyses were performed using a SPSS 13.0 program for Windows. A P-value < 0.05 was considered statistically significant.

RESULTS

The maternal findings (pregnancy day, water and food consumption, initial and final weights) of pregnant rats of controls and treated subcutaneously 100 and 200 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 pregnant rats. But we found that statistically significant decrease of body weight gain in OP250 treatment group than control and OP100 treatment group. And we found that statistically significant decrease of water

consumption in OP100 and OP250 treatment groups than control. Food consumption is significantly decreased in only OP250 treatment group from the control group.

The number of offsprings are presented in Table 2. Infanticidal behavior was observed in OP100 and OP250 treatment groups. It was significantly increased than control group. 47% of live offspring were killed and consumed in OP100 and 53% of live offspring were killed and consumed in OP250.

DISCUSSION

The present study was performed to investigate the morphologic and behavioral effects of pregnant rats exposed maternally to 4-tert-Octylphenol. The maternal body weight gain can be influenced by developmental effects (reduced litter size and reduced fetal weight), thus, when the developmental toxicity is absent alterations in this parameter can be interpreted as an indicator of maternal toxicity. The results of this study demonstrated that OP exposure reduced the body weight gain during treatment at 250mg/kg octylphenol.

Blake and Boockfor's studies [11] demonstrated a lot of alterations in histological structure of normal tissues belong to reproductive system. Some important differences were found in histopathological analyses of adrenal, pituitary gland, pancreas, thyroid and parathyroid tissues [6]. These findings are parallel to the studies by Saruhan and Özdemir [28]. Saruhan and Özdemir concluded that degeneration and deformation in endocrine tissues. Rosol et al. [29] demonstrated that the adrenal gland affected by chemically induced lesions. And Aydoğan and Barlas [5] concluded that many hormone levels were decreased, by octylphenol treatment to male rats. For this reason steroid hormones could effect to dams' behavior.

Babicky and Novakova [21] has examined diet and infanticide. They found that malnourished mothers committed infanticide during the weaning period. In present study, we found that food consumption is decreased in OP250 treatment group and water consumption is decreased in OP100 and OP250 treatment group from the control group. Food and water consumption may cause infanticidism in rats.

We found that 47% of live offspring were killed and consumed in OP100 and 53% of live offspring were killed and consumed in OP250. Our findings of this study are similar to the studies by Schardein et al. [19]. They found that 21% of live congenitally malformed offspring were killed and consumed, contrasted to only 3% of normal live offspring. Regarding stillborn offspring, mothers consumed 63% of the congenitally malformed ones, and 19% of the normal ones.

Several studies have examined relations between malformation and infanticide. Helander and Bergh [23], DeSantis and Schmaltz [18] found that congenitally malformed offspring were killed and consumed. Göktekin and Barlas [6] suggested that octylphenol exposure the during pregnancy period, has a negative histopathological effects. In this study, histopathologically malformed pups may cause infanticidal effects.

It was exhibited that octylphenol exposure in pregnant rats has a negative effect on their behavior. Octylphenol exposure during the pregnancy period to rats, although the amount of permeant to embriyo/juvenile is indeterminate. The results show that the embriyo/juvenile was treated by an affectional dose of octylphenol and was affected negatively. As Aydoğan and Barlas emphasized in their study [5], octylphenol exposure in foetal period may have a negative effect on adulthood reproductive system of male rats. Göktekin and Barlas [6], concluded that octylphenol administration during pregnancy cause negative impacts on the behavior of pregnant rats.

In conclusion, findings of this study demonstrate that octylphenol which mimics the estrogen caused adverse effects on pregnant rats. Although in the present study, the exact amount of octylphenol that passes from dam to pups is not clear; our findings suggest that the amount of octylphenol that has passed through placental barrier is enough to adversely effect pups.

In summary, our findings of this study indicate that exposure of octylphenol at doses of 100 mg/kg/day and 250 mg/kg/day subcutaneously during foetal life may cause adverse effects on behavior of pregnant Wistar albino rats after birth.

Table 1. Food and water consumption and maternal findings of dams exposed to octylphenol during pregnancy period [6].

Parameters	Octylphenol (mg/kg per /day)		
	Control	100	250
No.of dams	6	7	7
Pregnancy day	21.2±0.2	21.6±0.7	22.2±0.5
Food consumption (g/day)	20,4±1,13	17,2±0,85	16,2±1,06*
Water consumption (ml/day)	43,0±2,38	32,7±1,25*	33,0±1,01*
Initial body weights of pregnant (g)	186.7±5.9	188.6±10.2	208.6±6.6
Final body weights of pregnant (g)	268.0±16.9	259.7±16.7	258.8±8.2
Gain of weight (g)	81.3±3.7	71.0±8.5	50,2±6.6
Gain of weight %	%30.83	%27.33	%19.34*

Data are mean±S.E.

*Significantly different from control group, at P < 0.05.

Table 2. Incidence of dead infants after octylphenol treatment.

Parameters	Octylphenol (mg/kg per /day)		
	Control	100	250
Number of total offsprings	39	53	56
Number of dead offsprings	10/39	25/53	30/56
Number of live offsprings	29/39	28/53	26/56
% infanticide	25,64	47,17*	53,57*

*Significantly different from control group, at P < 0.05

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