Reproductive Toxicology 32 (2011) 298–303

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

In utero and lactational exposure to fenvalerate disrupts reproductive function in female rats

Marina T. Guerra [a,*], Fabíola C. de Toledo [a], Wilma De G. Kempinas [b]

[a] Graduate Program in Cell and Structural Biology, Institute of Biology, State University of Campinas – UNICAMP, Campinas, SP, Brazil
[b] Institute of Biosciences, UNESP – Univ Estadual Paulista, Department of Morphology, Botucatu, SP, Brazil

ARTICLE INFO

Article history:
Received 10 March 2010
Received in revised form 27 June 2011
Accepted 5 August 2011
Available online 25 August 2011

Keywords:
Fenvalerate
Female reproduction
Toxicology
Estrous cycle
Ovaries

ABSTRACT

Fenvalerate is a synthetic pyrethroid insecticide used in agriculture and domestic insect control. Some studies have proposed that it may act as an environmental estrogen; other studies suggest possible genotoxicity in germ cells. This study aimed to evaluate the effects of fenvalerate on the female reproduction in rats whose mothers were exposed during gestation and lactation. Pregnant Wistar rats were exposed to fenvalerate (40 mg/kg) or corn oil (vehicle) orally from gestational day 12 until the end of lactation. The dose selection was based on previous studies, whereas this was considered an effective dose. Results showed decreases in ovarian weight, pre-antral follicles and corpora lutea at PND 75 and an increase in the resorption number, when fertility test was performed at PND 80. Under some experimental conditions, fenvalerate may impair reproductive development of female offspring, manifested as reduced fecundity and ovulation number, resulting from the impairment in corpora lutea counting.
© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Some environmental chemicals have the potential to act as hormones or disrupt endocrine processes, leading to adverse health effects [1]. These endocrine disruptors [2–4] can interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones that responsible for the maintenance of development and homeostasis [5,6].

One example of those chemicals is the pyrethroid insecticide. Some recent reports indicate that pyrethroids, such as fenvalerate, are linked to endocrine disruption, subsequently leading to reproductive dysfunction [7].

Pyrethroid insecticides are the pesticides most commonly used to control agricultural and indoor pests [8]. Synthetic pyrethroids are analogs of the natural chemical moiety pyrethrin, which is derived from the pyrethrum plant species *Chrysanthemum cinerariaefolium* (Asteraceae) [9]. Exposure to pyrethroid insecticides has been associated with acute adverse reproductive effects and chronic exposure results in alterations in mammalian development [10–14]. Human exposure may occur occupationally or through residues in milk, fruits and vegetables [15–17].

Fenvalerate [4-chloro-α-(1-methylethyl)benzeneaceticacid cyano(3-phenoxyphenyl) methyl ester] belongs to the class denominated type II (displaying a cyano group at the carboxyl alpha position) of synthetic pyrethroids. Although fenvalerate has been shown to be more toxic to insects than mammalian species [18], is know that it can cause severe neurotoxic effects [19–21], through acting directly on the axon by interfering with the sodium channel gating mechanism [20,22,23].

Studies with fenvalerate in animal models have not revealed a unifying effect on development and reproduction. Several studies reported neither estrogenic nor anti-androgenic effects of this pyrethroid [24–30]. Some reports have shown a link to endocrine disruption, consequently leading to reproductive dysfunction [7]. Several synthetic pyrethroids, such as sumithrin, fenvalerate, and alethrin, are able to induce *in vitro* estrogenic responses [31–33]. Still other studies have reported anti-estrogenic responses to fenvalerate at early lifestages, e.g. perinatal treatment in female rats [34]. On the other hand, Kunimatsu et al. [24] reported that treatment of rats with different doses of fenvalerate did not show hormonal responses *in vivo*, and similarly, Arena et al. [14] showed no estrogenic activity *in vivo* in immature female rats at doses of 0.4, 1, 4, 8 and 40 mg/kg-d of fenvalerate.

Several studies showed toxic effects of fenvalerate on male reproductive system [15,35–41], by inducing significant decreases in testicular weight, epididymal sperm counts, sperm motility, and marker testicular enzymes for testosterone biosynthesis [35]. There are reports on the genotoxic effects induced by fenvalerate in somatic and germ cells of animals [36,40,42], such as increases in

* Corresponding author at: Department of Morphology, Institute of Biosciences, UNESP, Distrito de Rubião Jr s/n, Botucatu, São Paulo CEP 18618-000, Brazil. Tel.: +55 014 3811 6264; fax: +55 014 3811 6264.
E-mail addresses: trevizan@ibb.unesp.br, marinatguerra@hotmail.com (M.T. Guerra).

0890-6238/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2011.08.002

the frequency of sex chromosome aneuploidy and numerical chromosome aberration [40], DNA fragmentation in spermatozoa [36] and poor semen in male workers exposed occupationally to fenvalerate [39]. However, some studies suggest that fenvalerate is not genotoxic [43,44]. The effects of fenvalerate on female reproductive system are less understood [34,45].

The discordant effects of fenvalerate on reproductive parameters may, in part be due to different sources and purities of the compound or to different substances employed as vehicle for administration. The present study has evaluated the possible action of fenvalerate on reproductive function for female rats exposed during intra-uterine and lactational life, at a dose of 40 mg/kg-d which, represents 1/5 of the lethal dose in female rats (LD_{50} = 200 mg/kg) [24]. Dose selection was based on previous studies from our laboratory in which 40 mg/kg of fenvalerate was toxic to testis and epididymis [14], and 80 mg/kg administered to female rats during gestation and lactation caused convulsive behavior, hyperexcitability and mortality in 45% of the dams. Based on these data, we decided to choose the intermediary dose. The beginning of the mother's treatment coincided with the critical period of reproductive system development of the offspring, which continues after birth [46,47].

2. Materials and methods

2.1. Animals and exposure

Adult female (60 days of age, $n = 20$, 10 per experimental group) and male (90 days of age, $n = 20$) Wistar rats were supplied from colonies under Specific Pathogen Free (SPF) conditions from the Multidisciplinary Center for Biological Research (CEMIB – Unicamp), Campinas, and maintained in the Small Mammal Biotherium of the Morphology Department at the UNESP Biosciences Institute, Botucatu (www.cemib.unicamp.br/english/strains.php). Animals were housed in polypropylene cages (43 cm × 30 cm × 15 cm) with laboratory grade pine shavings as bedding. Rats were maintained under controlled temperature (23 ± 1 °C) and lighting conditions (12:12-h photoperiod). Standard rodent chow and filtered tap water were provided *ad libitum*.

Two non-gravid female rats were mated with one male, during the dark portion of the lighting cycle, and the day of sperm detection in the vaginal smear was considered day zero of gestation (gestational day 0 – GD 0). The pregnant females were randomly assigned to the experimental groups (10/group) and housed individually in cages. At post-natal day 1 (PND 1), pups were weighed and the number per litter was reduced to eight, maintaining, preferentially, female offspring. The male pups were used in another study to determine the consequent reproductive developmental and immunotoxic effects due to fenvalerate exposure [48].

The experimental protocol followed the Ethical Principles in Animal Research of the Brazil College of Animal Experimentation and was approved by the Bioscience Institute/UNESP Ethics Committee for Animal Experimentation (protocol no. 09/05-CEEA).

Pregnant rats were parsed into two groups (treatment and control), first one given fenvalerate (96.8% purity, composed by a racemic mixture of the four isomers (2R, αR; 2R, αR; 2S, αR; 2S, αS) in equal proportions [49]; Sumitomo Chemical, Japan) at 40 mg/kg bw-d (T, $n = 10$) and the other, corn oil (vehicle) (C, $n = 10$), by gavage (oral route), from GD 12 until the end of lactation (PND 21) to evaluate the possible effects of the treatment on the female offspring.

Dams were weighed on alternate days, during the treatment period (GD 12 to PND 21) to calculate the volume of fenvalerate to be administered and to evaluate possible signs of maternal toxicity.

2.2. Pups

Pups were weaned on PND 21 and the respective mothers were euthanized by CO_2 inhalation followed by decapitation on the following day.

2.2.1. External signs of puberty onset

Beginning on PND 30, all females were evaluated daily for vaginal opening (VO). The day of complete VO was recorded. Starting from the VO day, daily vaginal smears were collected to detect the day of first estrus, characterized by the predominance of cornified epithelial cells [50].

2.2.2. Estrous cycle evaluation

On PND 60, in fenvalerate treated ($n = 37$ females/10 litters) and control ($n = 42$ females/9 litters) groups, the estrous cyclicity of female rats was assessed from cells from daily vaginal smears, collected over a period of 15 days, as described [51]. Every morning 10 μL of 0.9% saline was instilled into the vagina and subsequently aspirated. The material was observed under light microscopy and the estrous cycle phase was determined, via cytology, by the following characteristics: predominance of nucleated epithelial cells (proestrus); predominance of cornified epithelial cells (estrus); the presence of cornified and nucleated epithelial cells and leukocytes (metaestrus); predominance of leukocytes (diestrus). The total frequency of each phase for every rat observed in this period was used to calculate the total length of the proestrus, estrus, metaestrus and diestrus (in days) and the estrous cycle length.

2.2.3. Analysis of reproductive organs

At PND 75, 12 females rats ($C = 5$, $T = 7$), one per litter, were euthanized by CO_2 inhalation followed by decapitation, on estrus phase, and ovaries and uteri (with fluid) were collected, weighed on a precision balance, fixed in Alfac's solution, dehydrated in ethanol and embedded in paraplast. Three sections (5 μm) per animal, separated by 50 μm distance, were obtained, mounted on glass slides and stained with hematoxylin and eosin.

In each ovary, ovarian follicles and corpora lutea were counted in 3 sections per animal. Follicles were classified according to Guerra et al. [50]. Primordial and primary follicles were enumerated together; oocytes surrounded by a single layer of either squamous or cuboidal epithelial cells were included. Follicles were classified as preantral when containing 2–4 layers of granulosa cells with no antral space. Antral follicles were classified when containing three or more layers of granulosa cells and a clearly defined antral space. Characteristics of atretic follicles included pyknotic granulosa cells, disorganized granulosa cells, degenerating oocyte and detachment from the basement membrane.

In the uterus, the endometrial height was measured, in 3 sections per animal, using a light microscope. For this, a Leica microscope DMLB (100×) coupled to a digital camera and a PC with software Leica Q-win (version 3 for Windows™) were used. In each section, five different regions were analyzed, resulting in a total of 15 measurements per animal.

2.2.4. Sexual behavior

During the first estrus after PND 80, females from control ($n = 9$) and treated ($n = 10$) groups, one per litter, were used for the mating test. Rats were maintained in controlled temperature conditions under an inverted 12-h light–dark cycle, for at least seven days for acclimation, with food and water *ad libitum*. To evaluate female sexual behavior, sexually experienced male rats were allowed ten mounts on the female and the presence of lordosis was measured. Results were expressed as the lordosis quotient (LQ, number of lordoses/ten mounts × 100). All females were used only once.

2.2.5. Fertility

This analysis was performed by examining natural mating. Female offspring (PND 80, immediately after sexual behavior test) from control ($n = 16$) and treated ($n = 19$) groups were placed with sexually experienced males (1:1), for up to 3 sexual cycles (3 estrus), in the beginning of the morning, during a dark period of the cycle. At the end of the afternoon, males and females were separated and vaginal smears were collected, in which initial sperm detection was determined to be GD 0. On GD 20 females were euthanized by CO_2 inhalation followed by decapitation. After the uterus and ovaries were collected, the numbers of corpora lutea (by gross morphology), implantation sites, early or late resorptions, live fetuses and fetal weights were determined. Early resorption is defined as a conceptus in which it is not grossly evident that organogenesis has occurred; a late resorption is a fetus which it is grossly evident that organogenesis had occurred. A live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus not demonstrating marked to extreme autolysis. Fetuses with extreme autolysis are considered to be late resorption [52]. From these results, the following parameters were determined: the mating index: number of females with mating/number of cohabitated females × 100; the gestation rate: number of pregnant females/number of inseminated females × 100; implantation rate (efficiency of implantation): implantation sites/corpora lutea × 100; pre-implantation loss rate: number of corpora lutea − number of implantations/number of corpora lutea × 100; post-implantation loss rate: number of implantations − number of live fetuses/number of implantations × 100; sex ratio: number of male fetuses/number of female fetuses × 100 [48].

2.3. Statistical analysis

Values are expressed as mean ± SEM and medians ($Q_1 - Q_3$), according to the characteristics of each variable. For comparison of inter-group results, Student's *t*-test and Mann–Whitney test were utilized. Differences were considered significant when $p < 0.05$ and the litter was utilized as the statistical unit. The statistical analyses were performed by GraphPad InStat (version 3.02).

Download English Version:

https://daneshyari.com/en/article/2594024

Download Persian Version:

https://daneshyari.com/article/2594024

Daneshyari.com