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Effect of beta-cypermethrin exposure on embryo implantation in mice

Yong-jiang Zhou[a,*], Ji-hong Wang[b], Li-qing Wang[a], Sha Xiao[b], Xiao-dan Wang[b], Hua-ling Yan[c], Cheng-feng Li[c], Hui-quan Zhu[a]

[a] Maternal, Child and Adolescent Health, School of Public Health, Hainan Medical College, No.3 Yixueyuan Road, Longhua District, Hainan Province, 571199, PR China
[b] School of Public Health, Hainan Medical College, Yixueyuan Road, Longhua District, Hainan Province, 571199, PR China
[c] The Undergraduates Class of 2013 Entry, School of Public Health, Hainan Medical College, Yixueyuan Road, Longhua District, Hainan Province, 571199, PR China

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ABSTRACT

The aim of this study was to investigate the effect of β-CP on embryo implantation in mice. Forty female mice were randomly assigned to four groups of 10 mice each: one control group and three β-CP treated groups. The control group was administered corn oil only, while the three β-CP-treated groups were given corn oil containing 5, 10, and 20 mg/kg bw d β-CP for 3 months through intragastric administration. The results indicated that the administration of β-CP decreased the rate of embryo implantation (all $p < 0.05$), E_2 level in the serum, and the expression of Homeobox A10 (HoxA10) protein. In addition, β-CP significantly increased ERa and PRA protein expression levels. These results suggest that β-CP can disrupt the balance of E_2 and P, influence ERa and PRA expression and their downstream-related molecule Hoxa10, and decrease embryo implantation.

1. Introduction

In recent years, pyrethroid pesticides (PPs) have been used for several purposes such as controlling weeds, insects, and vectors. Cypermethrin, a synthetic PP compound, is used as an insecticide in both agriculture and veterinary medicine applications [1–3], and beta-cypermethrin (β-CP) is widely used in China [2,3] and other countries [4]. Reports have indicated that in recent years, pyrethroid residue has been extensively detected in soil, urban areas, and agricultural streams, which poses a potential risk to humans [5]. In mammals, cypermethrin can accumulate in body fat, skin, the liver, kidneys, adrenal glands, ovaries, lungs, blood, and the heart [6,7]. The literature has indicated that cypermethrin induces alterations in serum proteins and lipid profiles, and damages kidney and liver functions [8,9].

β-CP has been demonstrated to be an endocrine-disrupting chemical (EDC), which can interfere with the reproductive process and lead to endocrine disruption in adolescent mice [10,11]. Some studies have also shown that cypermethrin adversely affects the male reproductive system of laboratory animals. Previous reports have shown that cypermethrin administration leads to a decrease in the serum levels of estradiol and progesterone in rats, and a significant decrease in the serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) in male rate [12]. Studies have also suggested that exposure to cypermethrin disrupts the sexual behavior of adult male NMRI mice. A study by Yousef et al. indicated that treatment with cypermethrin caused significant decreases in ejaculate volume, sperm concentration, and total sperm output and motility [13]. Oral administration of cypermethrin has been shown to impair the structure of seminiferous tubules, spermatogenesis, and androgen receptors in male rats [14]. Maternal cypermethrin exposure during lactation also permanently impairs testicular development and spermatogenesis in male offspring [15]. The literature has also reported antagonistic effects of cypermethrin on interleukin (IL)-6-induced androgen receptor activation [16]. Additionally, cypermethrin administration affects ovarian structure and functions, as it elevates follicular atresia and significantly lowers estradiol levels, dependent on time [17].

Early pregnancy loss, defined as occurring during the peri-implantation period before the pregnancy is recognized clinically, is a relatively common phenomenon in humans [18]. Implantation failure is the major cause of lost pregnancies, accounting for

* Corresponding author at: Hainan Medical College, No.3 Yixueyuan Road, Longhua District, Hainan Province, 571199, PR China.
E-mail address: Zhouyongjiang23@163.com (Y.-j. Zhou).

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Table 1
Experimental protocol.

Groups	Dose of β-CP (mg/kg bw d)	No. of animals	Administration method	Duration of exposure (months)	Treatment (at the end of exposure)	GD5 (8:00 am)
Control group	0	10	Intragastric administration: corn oil	3	Weighing the mice. Mating with male mouse (3:1). Emergence of vaginal plug as GD0.	Collection of blood samples. Injection of trypan blue. Investigation of implantation site. Tissues sampling and trimming.
5 mg/kg bw d group	5	10	Intragastric administration: corn oil + beta-CP	3		
10 mg/kg bw d group	10	10	Intragastric administration: corn oil + beta-CP	3		
20 mg/kg bw d group	20	10	Intragastric administration: corn oil + beta-CP	3		

approximately 75% [19,20]. Hence, embryo implantation is a key factor of early pregnancy [21]. The process of embryo implantation in mice is divided into three steps: pre-implantation on day GD4 (GD = gestation day), peri-implantation on GD5, and post-implantation on GD6 [22]. An abnormality in this process may cause an adverse outcome in the pregnancy [23]. Embryo implantation also involves the intimate interaction between an implantation-competent blastocyst and a receptive uterus, which occurs in a limited time period known as the "window of implantation" [20]. Endometrial receptivity is maintained only for a limited time, and is defined as the implantation window, which occurs on approximately day 5 (GD5) of pregnancy in mice [24–26]. Endometrial receptivity is regulated by ovarian steroids, estrogen (E_2), progesterone (P_4), estrogen and progesterone receptors and their downstream effectors [27]. The literature has reported that E_2 is a critical determinant for specifying the duration of the window of uterine receptivity for implantation [20]. P4 is essential for pregnancy maintenance in all mammalian species [26]. E_2 acts primarily through nuclear estrogen receptors (ER, mainly ERa but not ERβ) [23,28]. P_4 acts primarily through its cognate nuclear receptors, which are the progesterone receptors (PR, mainly PRA but not PRB) [29]. The downstream effector of estrogen and progesterone receptors, Homeobox A10 (Hoxa10), can regulate the proliferation and differentiation of stromal cells in the mouse endometrium to modulate endometrial receptivity [30].

However, the effects of β-CP on the female reproductive system have rarely been reported. Hence, this research focused on the toxic effects of β-CP on the reproductive function of female animals. The goal of this study was to investigate the effects of β-CP exposure on embryo implantation in female mice and to examine the expression levels of the genes and molecules involved in embryo implantation.

2. Materials and methods

2.1. Animals and reagents

Forty SPF weaned female Kunming mice (21 days old), with weights ranging from 13 to 15 g, were purchased from the Changsha Tianqin Biological Technology Company (Changsha, China) [Certificate No.: SCXK (XIANG) 2014-0011]. All experimental procedures were approved by the Ethical Committee of Hainan Medical College (Hainan, China). The mice were housed in plastic cages containing shavings as bedding material (poplar shavings) and were exposed to a 12 h light/12 h dark cycle at a constant temperature (22 ± 2 °C) and humidity (50%), with access to a standard chow diet (metal contents of diet, in mg/kg dry weight were Cu 10.0, Zn 15.0, Mn 56.0, Co 4.0, Fe 76.0), and water ad libitum. The mouse feed and bedding material were also purchased from the Changsha Tianqin Biological Technology Company (Changsha, China).

β-CP (Catalog No. 118-81-7, 99% pure) was purchased from Xiya Reagent (Shandong, China). The Hoxa10 primary antibody (Catalog No.: sc-28602) and the PRA primary antibody (catalog No.: sc-538) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The primary antibody to ERa (rabbit, ab32063) was purchased from Abcam (Cambridge, MA, USA). Goat anti-rabbit immunoglobulin G (IgG, ZB-2301), an anti-rabbit streptavidin-peroxidase assay kit (SP-9001), and a DAB (diaminobenzidine) kit (ZL-9018) were all purchased from ZSGB Bio (Beijing, China). A mouse estradiol and progesterone ((PROG) enzyme-linked immunosorbent assay (ELISA) kit (CK-E20376 M)) was purchased from Yan Hui Biological Technology Co., Ltd. of Shanghai, China. iQTM SYBR Green (170–8880) was purchased from Bio-Rad (Hercules, CA, USA). All primers were synthesized by Songon Biotech Co., Ltd (Shanghai, China).

2.2. Experimental design and animal treatments

As shown in Table 1, SPF Kunming mice were randomly assigned to four groups of 10 mice per group: one control group and three β-CP treated groups (5, 10, and 20 mg/kg bw d). Using intragastric administration, the control group was administered corn oil only, while the three β-CP treated groups were given corn oil containing 5, 10, and 20 mg/kg bw d β-CP. The dose of β-CP exposure used in this study was based on a previous study that was performed in Kunming mice [31]. The method of computing the solute weight and corn oil volume was as follows: the solute weight of the intragastric administration was calculated according to the average weight of the mice in each group during one week and the assigned concentration (5, 10, and 20 mg/kg bw d, respectively). The intragastric administration of the volume of corn oil in each group was calculated according to the average weight of the mice in each group during one week and the assigned concentration (0.01 mL/g, the volume of corn oil/mouse weight). The dose of intragastric administration was adjusted weekly. The mice in the four groups were weighed weekly.

After a three-month exposure period, the female mice from the four groups were weighed separately using an electronic balance. The weight of each mouse was recorded. A blood sample was collected from the inner canthus of each mouse from the four groups. Seven days after collecting the blood samples, estrous mice from each group were used for mating. During mating, one untreated male (weighing 80–120 g) and three females (1:3) were housed in a cage for one night, and the following day, the vaginal plugs of each female were examined (06:00) to confirm successful coitus. The day on which a vaginal plug was present was designated "GD0." To determine embryo implantation, female mice that had successful coitus were killed under diethyl ether anesthesia on GD5 between 08:00 and 09:00 after injection of trypan blue into the tail vein [26,32]. The mice that did not have successful coitus in the first estrous cycle continued to mate in the second estrous cycle. The mice that still did not have successful coitus in the second estrous cycle continued to mate in the third estrous cycle. The mice that had successful coitus in the three estrous cycles underwent the above-mentioned procedure. The mice that had still not had successful coitus at the end of the third estrous cycle were killed under diethyl ether anesthesia. Uteri were excised and photographed immedi-