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Beta-cypermethrin exposure affects female reproduction by enhancing oxidative stress in mice uterine tissue

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ABSTRACT

This study aimed to investigate the toxicity mechanism of beta-cypermethrin (beta-CYP) on fertility in female mice. Eighty female mice were randomly assigned to four groups of 20 mice each: one control group and three beta-CYP–treated groups. The control group was administered corn oil only, while the three beta-CYP–treated groups were given corn oil containing 1.38, 2.76, and 5.52 mg/kg bw.d beta-CYP for 180 days through intragastric administration. The results found that the 2.76 and 5.52 mg/kg bw.d beta-CYP significantly decreased the rate of successful pregnancy ($p < 0.05$). The concentrations of biomarkers related to oxidative stress were significantly elevated, while the concentrations of the endogenic enzymatic antioxidants were significantly decreased by the beta-CYP exposure (all $p < 0.05$). The expression levels of inflammatory-related molecules and the DNA-protein crosslink coefficient in mice uteri were significantly increased after beta-CYP exposure (all $p < 0.05$). The concentration of 8-hydroxy-2-deoxyguanosine was significantly increased in the 5.52 mg/kg bw.d beta-CYP group ($p < 0.05$). These results suggested that beta-CYP exposure significantly decreased female reproduction by enhancing oxidative stress in uterine tissue, which led to the increased inflammatory response and oxidative DNA damage in uterine tissue.

1. Introduction

Cypermethrin (CYP) is used as an insecticide in both agriculture and veterinary medicine applications (Li and You, 2015). Because of its wide use, its residues have been extensively found in the soil and in urban and indoor dust, which poses a potential risk to humans (Kuivila et al., 2012). Beta-cypermethrin (beta-CYP), a synthetic insecticide of type II pyrethroids, has been produced and used in agricultural pest control applications in China because of its high performance, safety, broad spectrum, and hypotoxicity to humans and livestock (Lu et al., 2009). It makes up more than 50% of the total pyrethroid market in China (Yang and Ji, 2015). The literature has reported that beta-CYP exposure can cause damage to multiple organ systems in humans and animals. Doses of 40 mg/kg beta-CYP can influence the hepatic energy metabolism in mice (Yuan et al., 2016). Male mice exposed to 20 mg/kg beta-CYP for 35 days had decreased sperm number and sperm motility and an intact acrosome rate, in addition to damage to the seminiferous tubules and sperm development (Wang et al., 2009). Our previous studies showed that beta-CYP treatment significantly affected the reproductive function of female mice and decreased embryo implantation (Zhou et al., 2018a, 2018b).

Oxidative stress is an important subject in pesticide toxicology. CYP treatment of 0.3 μg. L^{-1} has the potential to induce hepatic oxidative stress, DNA damage, and apoptosis in zebrafish (Jin et al., 2011b). Treatment with 10 and 20 mg kg^{-1} CYP significantly upregulated the mRNA levels of superoxide dismutase (SOD) and glutathione peroxidase in male mice (Jin et al., 2011a). Either single (170 mg/kg) or repeated (75 mg/kg per day for 5 days) oral administration of CYP was found to produce significant oxidative stress in cerebral and hepatic tissues of rats (Giray et al., 2001). After a dose of 10, 20, or 40 mg/kg CYP exposure, the kidney contents of reactive oxygen species (ROS), malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG), and DNA-protein crosslink (DPC) coefficients increased gradually in a dose-dependent manner, whereas glutathione (GSH) content decreased accordingly (Ma et al., 2014). CYP treatment (100 and 200 μM) reduced cell viability and induced apoptosis and increased ROS production and DNA damage in RAW 264.7 cells (Huang et al., 2016).

ROS are molecules containing at least one atom of oxygen and that have the potential to generate free radicals (Duhig et al., 2016). At the physiological level, they are involved in cell-signalling pathways and

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are important to cellular function (Pereira et al., 2015). The literature suggested that beta-CYP exposure can lead to oxidative stress. Exposure to beta-CYP at 4.57 mg/L could lead to significant differences in antioxidant biomarkers and lipid peroxidation in several tissues of common carp (*Cyprinus carpio*) (Stara et al., 2016). The beta-CYP treatments (10 and 20 mg/kg for 35 days) increased MDA and nitric oxide (NO) in testes of male mice, reduced the activity of catalase (CAT), glutathione peroxidase (GSH-Px), and SOD (Wang et al., 2009). Oxidative stress has been implicated in many reproductive and pregnancy disorders, from subfertility to miscarriage, maternal vascular disease, and preterm labour (Duhig et al., 2016). However, whether beta-CYP exposure affects oxidative stress in the uterine tissues of female mice has not been reported. Consequently, the current study aimed to examine the effects of beta-CYP exposure on oxidative stress in uterine tissue. Our study will lay the groundwork for further clarification of the female reproductive toxicity of beta-CYP.

2. Materials and methods

2.1. Animals and reagents

Eighty specific-pathogen-free (SPF) weaned female Kunming mice (21 days old) weighing between 13 and 15 g were purchased from Changsha Tianqin Biological Technology Company (Changsha, China; Certificate No. SCXK [(Yuan et al.)] 2014-0011). Mouse feed and bedding material were also purchased from Changsha Tianqin Biological Technology Company. Beta-CYP (catalogue No. 118-81-7, > 99% pure) was purchased from Xiya Reagent (Shandong, China). Mouse MDA enzyme-linked immunosorbent assay (ELISA) kit, mice NO ELISA kit, mouse GSH-PX ELISA Kit, mouse nitric oxide synthase (NOS) ELISA kit, mouse inducible NOS (iNOS) ELISA kit, and mice 8-OHdG ELISA kit were purchased from Yan Hui Biological Technology Co. Ltd. (Shanghai, China). Mice CAT ELISA kit was purchased from Wuhan Fine Biological Technology Co. Ltd. (Wuhan city, China). Mice ROS ELISA kit was purchased from Nanjing SenBenJia Biological Technology Co. Ltd. (Jiangsu province, China).

2.2. Experimental design and animal treatments

All experimental procedures involving animal care were carried out in accordance with the Guiding Principles for the Use of Animals in Toxicology, adopted by the Chinese Society of Toxicology. The mice used in this study were maintained in the experimental animal centre of Hainan Medical University in accordance with the institutional animal welfare policy. Adequate measures were taken to minimise the pain, discomfort, and stress of the animals. All experimental procedures were approved by the Ethical Committee. The mice were housed in plastic cages containing poplar shavings as bedding material, and the mice 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 (the metal content of the diet in mg/kg dry weight was Cu 10.0, Zn 15.0, Mn 56.0, Co 4.0, and Fe 76.0), and water was provided *ad libitum*.

As shown in Table 1, 80 SPF Kunming mice were randomly assigned to four groups of 20 mice each: one control group and three beta-CYP–treated groups (1.38, 2.76, and 5.52 mg/kg bw.d groups). The control group was intragastrically administered corn oil alone, while the three beta-CYP–treated groups were administered corn oil containing 1.38, 2.76, and 5.52 mg/kg bw.d beta-CYP for 180 days, respectively, until the completion of the study. The doses of beta-CYP in this study were based on our previous murine study (Zhou et al., 2018b). After a 180-day exposure period, 10 female mice from each group were weighed separately using an electronic balance. The weight of each mouse was recorded. The reproductive organ uteri were also then weighed on an electronic balance. Organ coefficients of the uteri were measured (organ coefficient = uterus organ weight/body weight x 100). The partial uterine tissues that were taken from each mouse of all

Table 1
Experimental protocol.

	No. of animals		Dose of beta-CYP (mg/kg.bw.d)	Administration method	Duration of exposure (days)	Day of treatment	Treatment (at the end of exposure)	GD5 (8:00 a.m.)
Control group	20	10	0	Intragastric administration: corn oil	180	181	Mating to detect the pregnancy rate	Injection of Trypan Blue Investigation of implantation site
		10	0	Intragastric administration:corn oil	180	181	Uterine tissues sampling and trimming	–
1.38 mg kg.bw.d group	20	10	1.38	Intragastric administration:corn oil + beta-CYP	180	181	Mating to detect the pregnancy rate	Injection of Trypan Blue Investigation of implantation site
		10	1.38	Intragastric administration:corn oil + beta-CYP	180	181	Uterine tissues sampling and trimming	–
2.76 mg kg.bw.d group	20	10	2.76	Intragastric administration:corn oil + beta-CYP	180	181	Mating to detect the pregnancy rate	Injection of Trypan Blue Investigation of implantation site
		10	2.76	Intragastric administration:corn oil + beta-CYP	180	181	Uterine tissues sampling and trimming	–
5.52 mg kg.bw.d group	20	10	5.52	Intragastric administration:corn oil + beta-CYP	180	181	Mating to detect the pregnancy rate	Injection of Trypan Blue Investigation of implantation site
		10	5.52	Intragastric administration:corn oil + beta-CYP	180	181	Uterine tissues sampling and trimming	–