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Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress

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Abstract

Cypermethrin (CYP), an insecticide, has deleterious effects on male reproductive function. The objective was to identify whether the effects of beta-CYP on male reproductive organs were associated with oxidative stress. Three doses of beta-CYP (1, 10, and 20 mg/kg) were administered to male mice for 35 d, with or without vitamin E (20 mg/kg). The moderate (10 mg/kg) and high (20 mg/kg) doses of beta-CYP not only decreased body weight and the weight of the testes, epididymides, seminal vesicles, and prostate ($P < 0.05$) but also reduced serum testosterone concentration and the expression of steroidogenic acute regulatory protein ($P < 0.05$), in addition to damaging the seminiferous tubules and sperm development. Furthermore, moderate and high doses of beta-CYP administration decreased sperm number, sperm motility, and intact acrosome rate ($P < 0.05$). Based on ultrastructural analyses, high doses of beta-CYP produced swelling and degeneration of mitochondria and the smooth endoplasmic reticulum of Leydig cells and caused the formation of concentric circles. These toxic effects of beta-CYP may be mediated by increasing oxidative stress, as the moderate and high doses of this compound increased malondialdehyde and nitric oxide in testes ($P < 0.05$); reduced the activity of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase ($P < 0.05$); and activated ERK1/2 ($P < 0.05$). Vitamin E reversed the effects of beta-CYP on testosterone production and testis damage ($P < 0.05$ vs. the high-dose group). Therefore, we inferred that beta-CYP damaged the structure of testes and decreased sperm output by inducing oxidative stress.

Keywords: Beta-cypermethrin; Oxidative stress; Pyrethroid insecticide; Reproductive function; Testis

1. Introduction

Cypermethrin (CYP) is a synthetic pyrethroid insecticide that has been widely used over the past 30 yr in China and other countries against pests, particularly Lepidoptera, cockroaches, and termites. In animals, cypermethrin has been used as a chemotherapeutic agent against ectoparasite infestations [1]. Beta-CYP is a mixture of the alpha and theta forms of the insecticide. Its activity is lower than that of alpha-CYP but higher than other CYPs. Beta-CYP has been applied widely for agricultural pest control in China and comprises more than 50% of the total pyrethroid market production [2]. Although cypermethrin was considered safe and was widely used on agricultural crops and forests as well as in public and animal health [3], there is accumulating evidence that chronic exposure or high-dose CYP has toxic effects on humans and animals.

Cypermethrin can be found in trace amounts or at higher concentrations in soil and air. In mammals, CYP can accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, lung, blood, and heart [4–6]. However, the main target for CYP is the central nervous system.

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Symptoms of CYP toxicity in laboratory animals include pawing, burrowing, salivation, tremors, writhing, and seizures. In humans, high doses of CYP result in twitching, drowsiness, coma, and seizures [7]. Cypermethrin exerts its neurotoxic effect through voltage-dependent sodium channels and integral protein ATPases in the neuronal membrane [8,9].

In addition to neurons, reproductive organs are another toxic target of CYP [10,11]. Cypermethrin decreases the weight of testosterone-sensitive organs, increases the height of seminal gland epithelium, and reduces sperm count and motility in male mice [11–15]. Moreover, CYP significantly reduced serum concentrations of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) [11], in addition to decreasing the number of implantation sites and viable fetuses in females mated with these male mice [11]. Taken together, it is evident that CYP disrupted male reproductive function.

The mechanism by which CYP affects male reproduction is unclear: Pyrethroids are rapidly metabolized in mammals, and several studies have shown that CYP damages the brain, liver, and erythrocytes by causing oxidative stress [16–18]. However, there are no studies that have investigated how oxidative stress mediates CYP-induced deficits in male reproduction. Consequently, the current study examined the role of oxidative stress in beta-CYP–induced damage to the testes and the possible protective effects of vitamin E. Vitamin E is a fat-soluble vitamin with potent antioxidant properties that scavenges intracellular free radicals and maintains cell membrane integrity by inhibiting lipid peroxidation induced by reactive oxygen species (ROS) [19].

2. Materials and methods

2.1. Materials

Beta-CYP (>99% pure) was obtained from Nanjing Panfeng Chem Ltd. (Nanjing, Jiangsu, China). The steroidogenic acute regulatory protein (StAR) antibody was kindly provided by Professor D.M. Stocco (Texas Tech University Science Center, Lubbock, TX, USA), and antibodies for phosphorylated (p)-ERK (E-4) and ERK were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The ^{125}I-testosterone radioimmunoassay (RIA) kit was purchased from the China Institute of Atomic Energy (Beijing, China). Total (Cu-Zn and Mn) superoxide dismutase (T-SOD) assay kit, malondialdehyde equivalents assay kit, nitrite assay kit, total nitric oxide synthase (NOS) assay kit, and specific NOS assay kit were purchased from Nanjin Jiancheng Biotechnology CO, Ltd (Nanjing, Jiangsu, China). Glutathione peroxidase (GSH-Px) assay kit and catalase (CAT) activity assay kit were purchased from Beyotime Institute of Biotechnology, China (Shanghai, China). All other reagents used in the analyses were of analytical grade and were obtained locally.

2.2. Animals and experimental design

Kunbai male mice (outbred strain) weighing ~30 to 35 g were provided by a local veterinary research institute. All animal treatment procedures were approved by the Animal Care Committee of Southwest University. These animals were adapted to the laboratory conditions before experiments and were housed in a standard animal facility under controlled temperature (22 °C), relative humidity (50% to 60%), and photoperiod (12 h light/12 h dark), with ad libitum access to water and food pellets.

According to the doses used by others [5,15] and a preliminary test, the highest dose in the study was 20 mg/kg body weight. Sixty adult Kunbai male mice were randomly allocated into six groups (N = 10/group). Doses were determined according to body weight, which did not differ between groups, and delivered periorally every day for 35 d. Both beta-CYP and vitamin E were dissolved in 0.1 mL peanut oil and administered to animals by gavage. Groups were as follows: (I) vehicle control: 0.1 mL (peanut oil); (II) low dose: 1 mg/kg beta-CYP; (III) moderate dose: 10 mg/kg beta-CYP; (IV) high dose: 20 mg/kg beta-CYP; (V) 20 mg/kg beta-CYP + 20 mg/kg vitamin E; and (VI) 20 mg/kg vitamin E.

At 36 d after the start of treatment, all mice were anesthetized with halothane and killed by aseptically severing the neck vessels.

2.3. Hormone assay

Blood samples were taken from the eye sockets of animals under anesthesia using a 1-mm syringe. Blood samples were centrifuged at 6111 × g for 4 min, and serum samples were stored at–70 °C until analysis. Serum hormone concentrations were assayed using RIA, as per kit instructions. Sensitivity (0.01 ng/mL) and coefficients of variation (intra-assay 5%, interassay 9%) were regarded as satisfactory.

2.4. Ultrastructure of Leydig cells and histologic structure of testes and epididymides

Samples of testes and epididymides were immersion-fixed in Bouin's solution for histopathology and