Toxicology Letters 287 (2018) 1–9

Contents lists available at ScienceDirect

Toxicology Letters

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

Alteration of mice cerebral cortex development after prenatal exposure to cypermethrin and deltamethrin

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ARTICLE INFO

Keywords:
Pyrethroids
Cerebral cortex
Prenatal
Cell cycle
Progenitor

ABSTRACT

Pyrethroids, a group of insecticides with high efficiency, low toxicity and wide spectrum, are used for pest control in agriculture. Here, we administered two representative pyrethroids (cypermethrin and deltamethrin) and an equal volume of vehicle (corn oil) to the pregnant ICR mice. This study investigated the effects of cypermethrin and deltamethrin on cerebral cortex development in mice as well as possible mechanisms in proliferation and differentiation. The results showed that histopathologic change did not occurred in the cerebral cortex using Hematoxylin and Eosin staining, however, the observation of fetuses exposed to cypermethrin and deltamethrin revealed reduction of neuronal proliferation, maturation and differentiation. Moreover, cypermethrin/deltamethrin-induced apoptosis of nerve cell was significantly higher in treated groups than that in control group by using flow cytometry, Western blot and TUNEL. It was worth mentioning that the newborns exposed to cypermethrin and deltamethrin did not showed abnormal neuronal distribution. These findings suggested that prenatal cypermethrin and deltamethrin exposure impaired corticogenesis.

1. Introduction

Chemically synthesized pyrethroids, similar to the natural pyrethroids because of their chemical structure, can affect the function of organs through neurotoxicity (Crago and Schlenk, 2015), endocrine disruption (Jin et al., 2015), abnormal development (Jin et al., 2009) and reproductive toxicity (Wang et al., 2009) in animals. It is now widely used in agricultural pest control and household pest cleanup. The pyrethroids can be classified into type I and type II, and cypermethrin (CP) and deltamethrin (DM) are common pyrethroid II pesticides used worldwide in agriculture, home pest control and disease vector control. In addition, they are considered as an insecticide closely related to human health and food safety (Jin et al., 2015; Singh et al., 2012).

CP and DM accumulate in soil, and traces of them may appear in vegetables, tea, fruits and other foods. CP and DM also have stomach toxicity and brain toxicity (Ncir et al., 2017; Singh et al., 2012). Although long-term exposure to low dose of CP is not enough to cause obvious symptoms of poisoning, the potential damage for reproduction cannot be ignored because of accumulation (Muangphra et al., 2015). CP and DM cause morphometric and structural changes in the genital organs by reducing the number of follicular cells, oocytes and corpora lutea through dose-dependent effects (Marettova et al., 2017; Petr et al., 2013). The previous studies showed that relative toxic potency of six individual pyrethroids for cortical neurons was followed by beta-cyfluthrin, lambda-cyhalothrin, deltamethrin, cypermethrin, bifenthrin and permethrin by disrupting voltage-gated sodium channels and altering cell excitability (Chen et al., 2017; Johnstone et al., 2017; Mohana Krishnan and Prakhya, 2016). In addition, pyrethroids were correlated with carboxylesterase metabolism in liver (Anand et al., 2006), and next generation sequence was used to identify differentially expressed genes for precise molecular mechanisms (Mamidala et al., 2012; Zimmer et al., 2017). Although DM and CP are widely used in human activities, the mechanism of them on cortical neurogenesis remain unclear, so we want to investigate the effect of DM and CP on neuronal progenitor proliferation, cell maturation, neuronal differentiation, apoptosis and neuronal migration in mammalian.

Our results demonstrated that CP/DM exposure inhibited the proliferation of neural precursor cells and neural stem cells, and promoted cell apoptosis in vivo and in vitro. The cell fate decision of newborn neurons was affected by CP and DM, respectively. These findings may be helpful for understanding the neurotoxicity mechanisms of pyrethroids.

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https://doi.org/10.1016/j.toxlet.2018.01.019
Received 12 December 2017; Received in revised form 20 January 2018; Accepted 24 January 2018

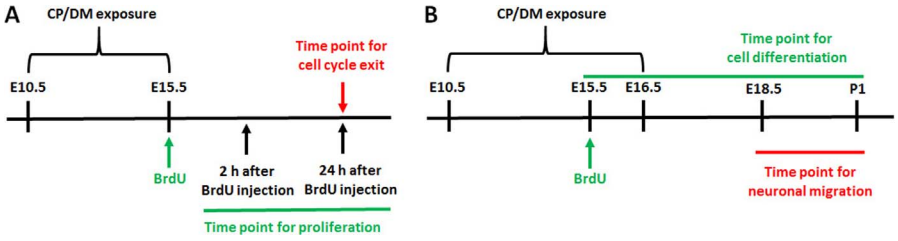

Fig. 1. Schematic diagram of the experimental protocol. (A) Experimental schedule to investigate the effect of CP/DM on cell proliferation of newly generated cells in the cerebral cortex. (B) Experimental schedule to study the effect of CP/DM on the neuronal migration.

A

Group	E16.5 fetuses				P1 newborns			
	No. of live fetuses per dam	Body weight(g.a)	Brain weight (g.b)	Relative(%,b/a)	No. of live fetuses per dam	Body weight(g.A)	Brain weight (g.B)	Relative(%,B/A)
Control	16.0±0.73	0.7254±0.1779	0.0520±0.0283	7.216±0.5210	13.5±0.56	1.4840±0.0555	0.0939±0.0030	6.337±0.1150
CP	11.0±0.73***	0.5831±0.3464**	0.0435±0.0033	7.505±0.4908	10.3±0.92*	1.4530±0.0427	0.0887±0.0068	6.111±0.4885
DM	11.2±1.30#	0.6094±0.0189##	0.0442±0.0047	7.219±0.6228	11.2±0.70#	1.4620±0.0360	0.0905±0.0034	6.190±0.2016

B

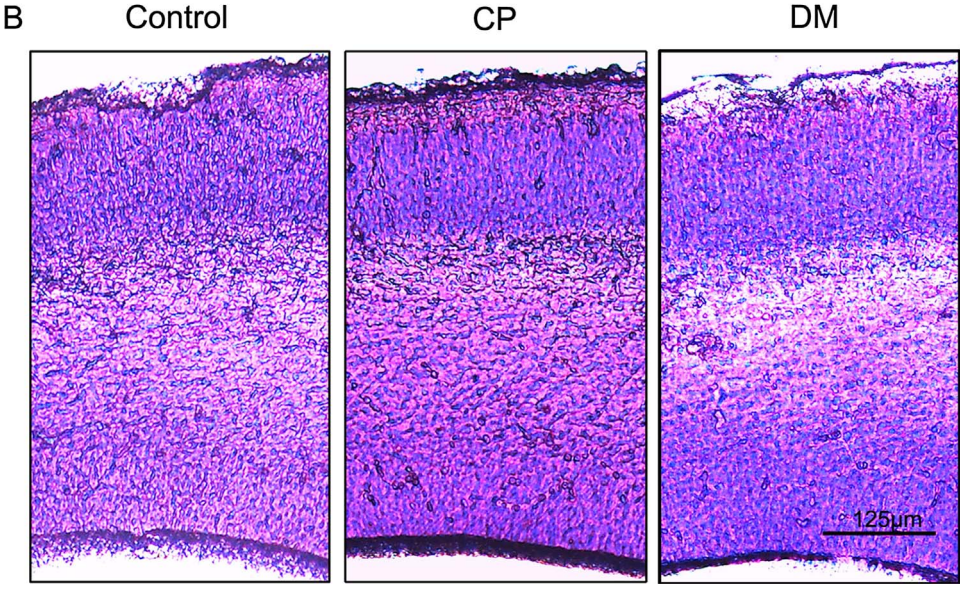

Fig. 2. The histopathological effects of CP/DM on embryos and its cortical development. (A) The number and the weight of live dams and offspring were counted in E16.5 and P1. CP/DM decreased survival rate of live fetuses, and there was significant difference in live body weight of dams between control mice and mice treated with CP/DM in E16.5. (B) There was no significant difference between treated groups and the control group by HE strain. Scale bar, 125 um.

2. Materials and methods

2.1. ICR mice

The mice were used and all procedures were performed according to the institutional guidelines for animal experiments. The day of vaginal plug detection was considered as gestation day 0.5 (E0.5), and the day of birth was designated as postnatal day 0 (P0). Schematic structure and the procedure in this study showed in Fig. 1, especially, purpose of the part A was to determine for cell proliferation and part B was to determine neuronal migration and apoptosis. All the animal experiments were performed according to the guidelines for the care and use of laboratory animals of Huaihe Hospital of Henan University.

2.2. Drug treatment

To assess the effect of CP/DM on cell proliferation in the VZ (Ventricular zone) and SVZ (subventricular zone) of cerebral cortex, mice were randomized into CP/DM and control groups (6 mice per group). Mice in each group then received intragastric administration of either CP/DM (1.2 mg/kg) or an equivalent volume vehicle (corn oil) from E10.5 to E15.5, and the dose was selected after observing toxicity signs with no death refer to previous studies (Cao et al., 2015; Ogaly et al., 2015). All animals were intraperitoneal (i.p) injected with 5-Bromo-2-Deoxyuridine (BrdU, 50 mg/kg) at E15.5 (Fig. 1), and sacrificed at 2 h after injection. All samples from each group were sacrificed and collected for cell cycle exit analysis.

To determine whether or not the CP/DM can affect neuronal migration at this dosage, the mice were randomized into control and CP/DM groups. Mice received intragastric administration of either vehicle or CP/DM from E10.5 to E16.5. Timed pregnant mice at E15.5 received a single intraperitoneal injection of BrdU (50 mg/kg) and mice were sacrificed at E18.5 or P1 after BrdU injection.

2.3. Tissue preparation

Postnatal mice were deeply anesthetized with sodium pentobarbital and perfused intracardially with 4% paraformaldehyde (PFA) in a 0.1 M phosphate buffer at a pH of 7.2-7.4. Brains were extracted and sections were sliced for 50 mm coronal sections. All brains were fixed overnight in 4% PFA at 4 °C for at least 24 h, embedded with O.C.T. (Sakura Finetek) on dry ice and ethanol slush.

2.4. Chemicals and antibodies

Deltamethrin (DM, CAS: 52918-63-5) and Cypermethrin (CP, CAS: 52315-07-8) were purchased from J&K chemical, China. BrdU (CAS: 59-14-3, Sigma) and Propidium Iodide (CAS: P4170, Sigma) were