Effect of Chlorpyriphos Exposure during Development on Skeletal and Smooth Muscles in Juvenile and Adult Rats

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***Abstract*—Chlorpyriphos (CPF) is a pesticide widely used in agriculture, commercial, and domestic applications. CPF acts in part through inhibition of acetylcholinesterase and thus can produce lasting effects on muscular system. However, the impact of chronic, low-dose exposure of CPF on mammalian muscles is poorly understood. In the present study, we examined in developing rats the effects of in utero and postnatal exposure to CPF, on contractile properties of the diaphragm, a respiratory skeletal muscle, and the digestive smooth muscles of the ileum. Wistar pregnant rats were administered by daily gavage from gestational day 1 to postnatal day 21 (PND21) with vehicle (control) or CPF at different doses: a low dose (1 mg/kg/d) and a high dose (5 mg/kg/d). At PND 21 and 60 the rats were sacrificed and both muscles of the ileum and the diaphragm were sampled for measurement. At PND60, there was a decrease in body weight of rats exposed to 1 mg/kg/d of CPF. Rats exposed to 5 mg/kg/d of CPF showed a lower body weight at all ages studied. In addition, CPF exposure increases the twitch tension of the diaphragm at PND 21 and 60. For the ileum, there was a significant increase in the twitch tension (g/ cm2) of the smooth muscle at PND21 in both groups. In conclusion, chronic prenatal and postnatal exposures to CPF affect the contractility of both the diaphragm and ileal smooth muscles. Further investigations are required to explain these increases in the contractility.**

Keywords-Pesticide; chlorpyriphos; perinatal life; muscle contractility.

I. INTRODUCTION

Pesticides are unique contaminants in that they are intentionally released into the environment to elicit toxicity in certain "pest" species. Unfortunately, a lack of selectivity often leads to problems of toxicity in humans and other non-target species. Organophosphorous (OP) pesticides are the major class of insecticides in the world today [1]. Poisoning with organophosphorous compounds is a global health problem. Organophosphorous compounds inhibit acetylcholinesterase resulting in accumulation of acetylcholine (Ach) and overstimulation of cholinergic synapses. Acetylcholine is an important neurotransmitter in both central and peripheral nervous system; in Central Nervous System (CNS), it is implicated in memory process and learning, and especially in muscle activity and vegetative functions; in peripheral nervous system (PNS), it is also implicated in the regulation of synaptogenesis [2]. The accumulation of the neurotransmitter acetylcholine causes hyperactivity in CNS and in neuromuscular junctions. Patients exposed to OP pesticides die mostly from respiratory failure and lung injury [3].

Chlorpyriphos (CPF), O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)- phosphorothioate, was one of the most widely used OP insecticides in the U.S [1]. From 2000, all residential uses were banned. In the European Union, CPF is one of the widely used pesticides in agriculture with moderate toxicity with a rat oral lethal dose 50 (LD50) of 135-163 mg/kg [4].

People are regularly exposed to a wide range of pesticides which are food contaminants. Studies on chlorpyriphos showed that prenatal CPF exposure was associated with intrauterine growth retardation and decreased birth weight [5][6][7][8], developmental neurotoxicity [9][10][11] after prenatal exposure and it is also associated with high risk of lung cancer and leukemia among agriculture workers [12].

Recent investigations have focused on the determination of the mechanisms behind the age-related toxicity differences, since juveniles are more susceptible [13], and on the alterations resulting from repeated exposure during development. Moreover, there are numerous studies in

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animals following acute [14] or chronic exposure to CPF [15] showing a significant inhibition of acetylcholinesterase activity in brain, diaphragm, liver, retina and blood cells and a decrease in muscarinic receptor (QNB) binding in brain, heart and retina during critical periods of development [16][17][18] or in adulthood [17].

Our objective was to investigate the effects of repeated gestational and postnatal exposure to CPF on the contractility of the respiratory muscle (diaphragm) and longitudinal smooth muscle of the ileum in Wistar rats and to determine the mechanism by which the contractility is affected. Such an investigation cannot be performed in humans. In this paper, we show the effects of chlorpyriphos exposure on body weight, survival rate, and the *in vitro* contractility properties of both diaphragm and longitudinal smooth muscle of ileum.

II. MATERIALS AND METHODS

The methods used in our experiments are summarized in the sections below.

A. Chemicals

Chlorpyriphos (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)- phosphorothioate, purity 99.8%) was purchased from LGC standards SARL (6, Rue Alfred Kastler, 83076, MOLSHEIM, France). It was dissolved in commercially available rapeseed oil (the vehicle) at dose of 1 mg/ml (for the CPF1 group) and 5 mg/ml (for the CPF5 group) in order to expose animals to 1 or 5 mg/ml/kg body mass/day. Other biochemicals were obtained from Sigma-Aldrich Chemical C (L'Isle d'Abeau Chesnes, 38297 Saint-Quentin Fallavier, France).

B. Experimental Design

Twelve female and 6 male Wistar rats (aged 8 weeks on arrival, body mass 250-300 g) were obtained from Janvier LABS (Le Genest Saint Isle Saint Berthevin, 53941 France). All animals were housed in cages in a controlled-temperature (23°C) room with a 12:12 h alternating light:dark cycle. They were maintained on a standard pelleted diet, with tap water ad libitum. After a 1-week acclimation period, females were mated with males and pregnancy was determined by the presence of spermatozoa in the vagina checked with a smear. After fertilization, female rats were individually housed in clean plastic cages and randomly assigned to a treatment group or control group. Pregnant rats were exposed by gavage from gestational day 1 (GD1) to the post natal day (PND21) at different doses: 1 mg/kg/d (CPF1) and 5 mg/kg/d (CPF5) vs vehicle. After weaning at PND21, pups were administered with the same doses of CPF until day 60 of age (PND60). Hence, pups were studied at two time points: weaning day (PND21) after gestational and lactational exposition and in adulthood (PND60) after further exposition to CPF in food.

C. Sample collection

At day 21, half of rats were euthanized with an intraperitoneal overdose of sodium pentobarbital (1 ml/kg; 200mg/ml solution) and muscles of both diaphragm and ileum were sampled for measurement of contractile properties. The second half of rats was weaned and administered with the same doses of CPF until day 60 of age (PND60). They were sacrificed and the contractile properties of their muscles studied.

D. Organ bath physiology

After euthanasia, the diaphragm and pieces of ileum were removed. Muscle strips were mounted in organ bath containing oxygenated Krebs solution at 37°C and pH 7.4. After 30min equilibration period, the ileal segments were stimulated by electrical field stimulation (EFS) (100 v- 32 Hz). For the diaphragm segments, fatigue was induced by application of a low frequency fatigue protocol consisting of supramaximal stimuli (2 Hz, 2 ms, 12 V) delivered directly to the muscle for 5 min for induction of fatigue.

E. Statistical analysis

Statistical analyses were performed with graphpad prism 5 software (graphPad software, Inc, San Diego, California) . If groups were significantly different in in a one-way ANOVA test, an impaired t test was then performed. Statistical significance is reported for the $p \leq 0.05$. Values were expressed as the mean±SEM.

III. RESULTS

Our results obtained are exposed in the sections below.

A. Effect of CPF exposure on body mass and mortality of pups

Figure 1 shows that at birth and PND21 there was no difference in body mass of rats exposed to CPF1 compared to controls. However, at PND60, body mass of CPF1 rats was significantly decreased. Body mass of rats exposed to 5 mg/kg/d of CPF was significantly lower than control at birth, PND21 and PND60.

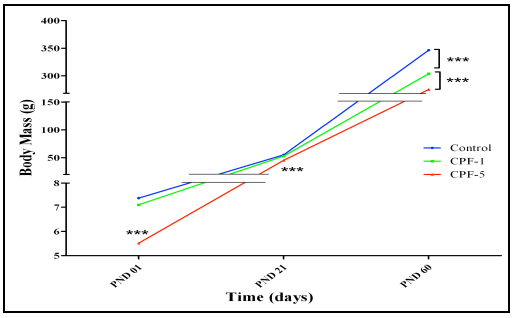

Figure 1. Change in body mass of rat pups during repeated oral exposure at PND01, PND21and PND60.

It should be noted that deaths occurred essentially between PND01 and PND21 in pups exposed during in utero and postnatal periods to CPF5 (Table I).

TABLE I. NUMBER OF DEATHS OCCURING DURING REPEATED ORAL EXPOSURE TO THREE DOSES OF CHLORPYRIPHOS FROM PND01 TO PND21.

DOSES	Control	CPF-1	CPF-5
PND 01-21	0/25 (0%)	0/25 (0%)	13/32 (40%)

B. In vitro muscle contractility

At PND 21 and PND 60, the diaphragm shows a significant increase in twitch tension after oral exposure to CPF at doses CPF1 and CPF5, as depicted in Figure 2.

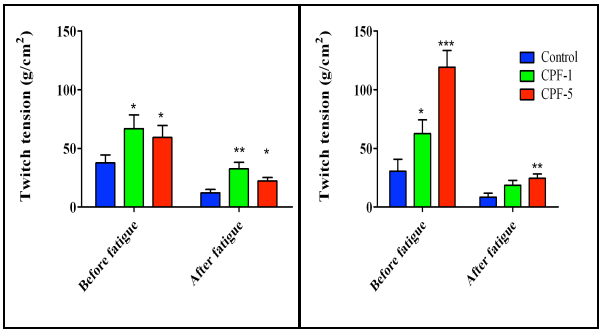

Figure 2. Twitch tension of diaphragm after oral exposure to CPF at PND 21 (2.a) and PND 60 (2.b). Values are expressed as mean ± SE (n=10/group) *significantly different from control (p<0.01).

However, only at PND60, the fatigability index was higher in CPF5 group compared to controls, as shown in Figure 3.

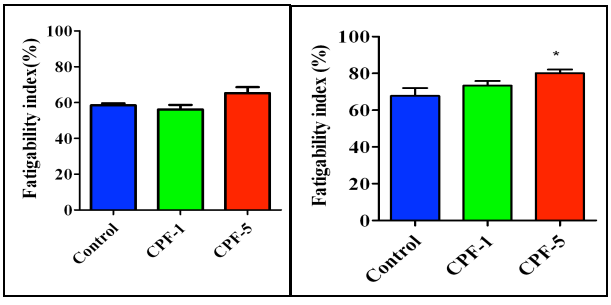

Figure 3. Effect of CPF exposure on fatigability of diaphragm at PND21 (fig.3.a) and PND60 (fig.3.b).

The EFS-stimulation of ileal segments of exposed rats showed a significant increase in the amplitude of contraction at PND21 and a significant decrease in the amplitude of contraction at PND60, as presented in Figure 4.

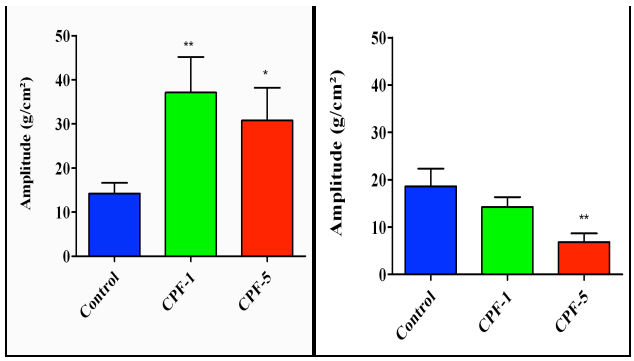

Figure 4. Contractility of ileal longitudinal smooth muscle at PND21 (fig.4.a) and PND60 (fig.4.b) in controls, CPF1 and CPF5.

IV. DISCUSSION

The data from this study show that in utero and postnatal chlorpyriphos exposure alters the contractility of diaphragm and smooth muscle of ileum. Organophosphorus (OP) insecticides elicit toxicity through inhibition of acetylcholinesterase, leading to accumulation of acetylcholine in the nervous system and consequent signs of cholinergic toxicity [19]. Our results show that perinatal exposure of rat pups to low dose CPF has an impact on growth, with lower body weight in CPF1 group at adulthood and in CPF5 group at birth, PND21 and PND60 as described by Mansour and Moussa [20].

This study was designed to investigate, for the first time, the effects of chlorpyriphos exposure from the first day of gestation to the post natal day (PND21) and to adulthood (PND60) on the contractility of diaphragm and smooth muscle of ileum. As expected, the exposure to CPF alters the contractility of diaphragm and ileal smooth muscle. At PND21 and PND60 the twitch tension of diaphragm was significantly increased in both CPF1 and CPF5 group. This can be explained by the inhibition of the acetylcholinesterase in the neuromuscular junction and will be confirmed by the dosage of acetylcholinesterase activity. Previous studies reported decreased acetylcholinesterase activity in diaphragm following repeated oral postnatal exposure to CPF from PND1 to PND21 [16] and in adult rats [14]. Furthermore, the fatigability index was higher in adult rats exposed to CPF5. This can be related to the protein content, the calcium intake or the neuromuscular junction structure. Further experiments are required to explain this increase. The amplitude of contraction of the ileal longitudinal smooth muscle of the ileum was increased in the rats exposed to both CPF1 and CPF5 doses at PND21. However, at PND60, the

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amplitude of contraction was decreased in exposed group. This can be explained by the difference of administration of CPF: at PND21, rats were exposed to CPF in utero and via lactation; the chlorpyriphos is mostly detoxified by the organism of the mother. After weaning up to day 60, intestinal tract is directly in contact with CPF. Those features may provide an explanation for the difference observed between the two age groups.

V. CONCLUSION

The chronic, low-dose oral exposure to CPF from day one of gestation to postnatal day 60 alters the contraction of skeletal respiratory muscle, and longitudinal ileal smooth muscle in juvenile and adult rats. These effects could alter the respiratory pattern and the intestinal transit which will be investigated in future. These findings suggest that the pesticides exposure should be avoided as much as possible. The mechanisms that lead to the increase in the contractility have to be investigated. This change in the contractility of the muscle can be related to modification of the muscle structure including muscle fibers distribution, myofibrillar protein and/or RNA content. Thus, further investigations are needed in order to explain these muscle alterations.

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