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Repeated exposures to chlorpyrifos lead to spatial memory retrieval impairment and motor activity alteration

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ABSTRACT

Chlorpyrifos (CPF) is one of the most commonly used insecticides throughout the world and has become one of the major pesticides detected in farm products. Chronic exposures to CPF, especially at the dosages without eliciting any systemic toxicity, require greater attention. The purpose of this study was, therefore, to evaluate the behavioral effects of repeated low doses (doses that do not produce overt signs of cholinergic toxicity) of CPF in adult rats. Male rats were given 0, 1.0, 5.0 or 10.0 mg/kg of CPF through intragastric administration daily for 4 consecutive weeks. The behavioral functions were assessed in a series of behavioral tests, including water maze task, open-field test, grip strength and rotarod test. Furthermore, the present study was designed to evaluate the effects of repeated exposures to CPF on water maze recall and not acquisition. The results showed that the selected doses only had mild inhibition effects on cholinesterase activity, and have no effects on weight gain and daily food consumption. Performances in the spatial retention task (Morris water maze) were impaired after the 4-week exposure to CPF, but the performances of grip strength and rotarod test were not affected. Motor activities in the open field were changed, especially the time spent in the central zone increased. The results indicated that repeated exposures to low doses of CPF may lead to spatial recall impairments, behavioral abnormalities. However, the underlying mechanism needs further investigations.

1. Introduction

Chlorpyrifos (O,O′-dithyl-O-3,5,6-trichloro-2-pyrydyl phosphorothionate, CPF) is one of the most widely used organophosphates insecticides (OPs) throughout the world. Although the United States Environmental Protection Agency (USEPA) has terminated indoor residential use since 2000, CPF is still one of the most widely used insecticides, and more than 8 million pounds of CPF are used each year for agricultural purposes in the United States (USEPA, 2011a, 2011b). CPF is also widely used in agriculture as the substitutes for methamidophos and parathion in China, and has become one of the major pesticides detected in farm products (Chen et al., 2009; Sun and Chen, 2008). Since agricultural uses on orchards and row crops persist, CPF has been frequently detected in air, food and water. Although various standards exist to minimize its exposure in food and water, CPF is frequently used and bio-accumulates in certain scenarios (Kale et al., 2002; Varó et al., 2002; Tilak et al., 2004; Jantunen et al., 2008). According to Gurunatban's study, CPF continued to accumulate on children's toys and hard surfaces 2 weeks after a single broadcast use of CPF in apartment rooms (Gurunathan et al., 1998). Wright et al. (1994) also reported the presence of CPF several years—as much as 2 and 8 years—after termite treatment of home foundations or concrete slabs. Although CPF has a half-life less than 60 days, the use of CPF around the world continues to produce detectable levels of CPF in the environment, thereby posing an ongoing risk for low-level exposure (Arcury et al., 2007; Davis and Ahmed, 1998; Gurunathan et al., 1998; Wright et al., 1994). People might be exposed to the same pesticide from different sources and have it accumulated in the body. Relatively less attention has been given to the subject of chronic, low-level exposures to CPF that are not associated with acute cholinergic symptoms. Chronic exposures to CPF, especially at the dosages that will not elicit any significant systemic toxicity, require greater attention and further study.

Animal studies have also shown that exposure to CPF can produce neurotoxic effect. Several animal studies have assessed the cognitive alterations after acute or chronic exposure to CPF in the behavioral tests, including Morris water maze, visual signal detection task, T-maze test, open-field test, 16-arm radial maze (Braquenier et al., 2010; Bushnell et al., 2001; Jett et al., 2001; Levin et al., 2001; Lopez-Crespo et al., 2009; Middlemore-Risher et al., 2010; Sanchez-Amate et al., 2001). In these studies, most of the attention had been paid to the prenatal or postnatal CPF exposures due to its developmental neurotoxicity, and the effect of CPF exposure on adult

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individuals has not been consistently considered. Some repeated studies in which adult animals were used as experimental subjects had contradictory results on the cognitive and behavioral effects (Mattsson et al., 1996; Maurissen et al., 2000; Moser et al., 2005; Samsam et al., 2005). It is difficult to form a consistent view on the cognitive effects of repeated CPF exposure in adults. Hence, the objective of this study was to evaluate the behavioral effects of repeated, "low-level" CPF exposures in adult male rats. Furthermore, the present study was designed to evaluate the effects of repeated exposures to CPF on water maze recall and not acquisition. The difference in the design from most of the other published studies may help us understand the behavioral effects of repeated, "low-level" CPF exposure. The low-level here was defined as the doses that do not produce overt signs of cholinergic toxicity, e.g. excessive salivation, urination, defecation, muscle fasciculation and so on (Ray and Richards, 2001; Middlemore-Risher et al., 2010). It should be noted, however, that the doses used in the present study are much higher than would be expected from incidental environmental exposures in general populations. These doses were used to model the types of exposure that may be experienced by agricultural and industrial workers, as well as pesticide applicators.

The acute toxic effects of OPs are caused by the accumulation of acetylcholine (ACh) in the synapses, resulting in the stimulation of cholinergic pathways. But the chronic, low-level exposure may not result in severe AChE inhibition, and the effects of daily contact with low level of OPs (especially environmental exposure) are not well understood (Abou-Donia, 2003). Some epidemiology studies had reported cognitive dysfunction, neuropsychological abnormality and behavioral alteration after repeated exposures (Jamal et al., 2002; Kamel et al., 2005; 2007; Mackenzie et al., 2010; Pilkington et al., 2001; Rohlman et al., 2007; Steenland et al., 2000; Stephens et al., 1995). Many experimental studies had found that repeated CPF exposures could induce alterations in animal behaviors (Chakraborti et al., 1993; Mattsson et al., 1996; Moser et al., 2005; Sanchez-Amate et al., 2001). Take into account the cognitive dysfunction, neuropsychological abnormality and behavioral alteration after repeated OPs exposure reported in the epidemiology studies discussed above, the results of daily exposure to low doses of CPF would be very important for the understanding of the foundlings reported in the epidemiology studies.

In the present study, we investigated the neurobehavioral effects of the repeated, low doses of CPF in adult male rats.

2. Materials and methods

2.1. Reagents and chemicals

CPF (99.5%) was purchased from the Institute for the Control of Agrochemicals, Ministry of Agriculture, PR China. All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO).

2.2. Animals and treatments

Male albino Wistar rats weighing 180–200 g were supplied by the Laboratory Animal Center, Academy of Military Science, PR China (Beijing, China). The rats were housed in a temperature-controlled (24 ± 1 °C) room and maintained on a 12 h light/dark cycle with food and water *ad libitum*. They were housed three or five in one cage for at least 1–2 week prior to dosing. All animal procedures were approved by the Institutional Animal Care and Use Committee at the Academy of Military Medical Sciences and were in strict accordance with the internationally accepted principles and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. CPF was dissolved in the olive oil vehicle and was administrated at dosages of 1.0, 5.0 and 10.0 mg/kg in volume of 1.0 ml/kg once daily for 4 consecutive weeks through intragastric administration, and the control animals received equivalent administrations of the olive oil vehicle on the same schedule. A total of 85 rats were randomly assigned into four groups (there are 22 rats in the 10.0 mg/kg group). Ten rats in each group were tested on water maze experiments and grip strength (randomly assigned), and the rest in each group were tested on rotarod and motor activity. The body weights of each rat and food consumption per cage were recorded on scheduled days. The rats were humanly anesthetized with pentobarbital sodium 24 h after the final CPF or vehicle administration. The hippocampi and blood of the rats from motor activity and rotarod test were isolated and randomly assigned for AChE assay. The rats used for water maze and grip strength test were used for another study.

2.3. AChE activity assay

Hippocampi were freshly isolated and immediately homogenized with ice-cold physiological saline. The homogenates were centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatants were kept for AChE assay. Cholinesterase (AChE) activity was measured spectrophotometrically by the modified DTNB assay (Ellman et al., 1961). Briefly, 30 μl tissue supernatants were added to 96-well plates containing the reaction mixture (3 mM acetylthiocholine iodide substrate and 1 mM 5,5′-dithiobis-2-nitrobenzoic in phosphate buffer at pH 7.4). Absorbance at 414 nm was recorded with a Multiskan microplate spectrophotometer (Thermo labsystems, Finland). Protein concentrations were also measured spectrophotometrically using the BCA protein assay (Boster, Wuhan, China). The erythrocyte AChE activity was also measured by the modified DTNB assay. To prepare erythrocytes AChE, rat venous blood was collected in a heparinized tube and centrifuged at 3000 rpm for 10 min at 4 °C. The erythrocytes were washed 3 times with ice-cold physiological saline followed by lysis in 50-fold volumes of sodium phosphate buffer 8.45 mM, pH 8.0. After 2 h stirring, the lysed cells were centrifuged at 12,000 rpm for 15 min at 4 °C. The erythrocyte ghosts were washed and diluted then added to a cuvette containing the reaction mixture described above.

2.4. Behavioral experiments

2.4.1. Morris water maze experiments

Modified Morris water maze experiments were conducted in a circular stainless steel pool with the inner surface painted black (height: 50 cm, diameter: 150 cm) (Kee et al., 2007; Morris et al., 1982; Remondes and Schuman, 2004). The pool was filled with clear water maintained at 24 ± 1 °C. Four starting positions were marked on the outer wall of the pool (I: north, II: west, III: south, and IV: east). During the acquisition experiments, a black escape platform (height: 38.5 cm, diameter: 10 cm) was submerged 1 cm below the surface of the water and fixed in the center of the west quadrant. The swimming activity of each rat was monitored by Videomex-V Image Motion System (provided by the Chinese Academy of Medical Sciences).

Before exposure to CPF, each rat was given four trials per day for 4 consecutive days (acquisition experiments). For each trial, the rat was placed into the water facing the pool wall in one of the four quadrants, and allowed to swim for 90 s to find the hidden platform. When the rat found the platform within 90 s, it was allowed to rest on the platform for 30 s. If the rat could not arrive at the platform within 90 s, it was guided onto the platform and given 30 s to spend on the platform. The daily orders of the starting positions were random for each rat on each day, so that all four quadrants were used once every day. Probe trials were carried out 16 h after the last CPF administration, in which the platform was removed from the pool. The rats were placed in the water maze in the east quadrant (IV) because the platform was in the west quadrant in the acquisition phase. The latency to find the former platform position (circular