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Spatial learning impairment in prepubertal guinea pigs prenatally exposed to the organophosphorus pesticide chlorpyrifos: Toxicological implications

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ABSTRACT

Exposure of the developing brain to chlorpyrifos (CPF), an organophosphorus (OP) pesticide used extensively in agriculture worldwide, has been associated with increased prevalence of cognitive deficits in children, particularly boys. The present study was designed to test the hypothesis that cognitive deficits induced by prenatal exposure to sub-acute doses of CPF can be reproduced in precocial small species. To address this hypothesis, pregnant guinea pigs were injected daily with CPF (25 mg/kg, s.c.) or vehicle (peanut oil) for 10 days starting on presumed gestation day (GD) 53–55. Offspring were born around GD 65, weaned on postnatal day (PND) 20, and subjected to behavioral tests starting around PND 30. On the day of birth, butyrylcholinesterase (BuChE), an OP bioscavenger used as a biomarker of OP exposures, and acetylcholinesterase (AChE), a major molecular target of OP compounds, were significantly inhibited in the blood of CPF-exposed offspring. In their brains, BuChE, but not AChE, was significantly inhibited. Prenatal CPF exposure had no significant effect on locomotor activity or on locomotor habituation, a form of non-associative memory assessed in open fields. Spatial navigation in the Morris water maze (MWM) was found to be sexually dimorphic among guinea pigs, with males outperforming females. Prenatal CPF exposure impaired spatial learning more significantly among male than female guinea pigs and, consequently, reduced the sexual dimorphism of the task. The results presented here, which strongly support the test hypothesis, reveal that the guinea pig is a valuable animal model for preclinical assessment of the developmental neurotoxicity of OP pesticides. These findings are far reaching as they lay the groundwork for future studies aimed at identifying therapeutic interventions to treat and/or prevent the neurotoxic effects of CPF in the developing brain.

1. Introduction

Chlorpyrifos (CPF) is the most commonly used organophosphorus (OP) pesticide in agriculture worldwide and continues to be the top-selling OP pesticide for residential use in various countries. It is anticipated that, in part because of the broad-spectrum pest control effectiveness and low cost of this pesticide, the global market of CPF will grow steadily between 2015 and 2022 (Grand View Research, 2015). Upon recognizing the detrimental effects of CPF to the developing brain, the United Stated (U.S.) Environmental Protection Agency banned in 2001 the residential use of this pesticide in the U.S. (Israel, 2012). Nevertheless, CPF could still be detected in 78% of American households surveyed between 2005 and 2006 (Stout et al., 2009). Thus, exposure to CPF will remain for years to come a serious public health concern in both agricultural and residential settings in the U.S. and throughout the world.

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Epidemiological studies have provided evidence that OP pesticides are toxic to the developing human brain (Rauh et al., 2012; Rosas and Eskenazi, 2008). Specifically, low birth weight and length in addition to increased prevalence of psychomotor and cognitive deficits among children ages 2–7 have been associated with prenatal exposure to CPF levels that produce measurable concentrations of the pesticide in maternal and/or umbilical cord blood but do not trigger overt signs of maternal intoxication (Perera et al., 2003; Rauh et al., 2006; Whyatt et al., 2004). Working memory deficits have also been observed among 7-year-old children prenatally exposed to sub-acute levels of CPF, with boys presenting more severe deficits than girls (Horton et al., 2012).

The acute toxicity of CPF results primarily from the irreversible inhibition of acetylcholinesterase (AChE), the enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh), and is characterized by a classical cholinergic crisis defined by miosis, profuse secretions, diarrhea, diuresis, muscle fasciculation, tremors, motor convulsions, and respiratory distress that can lead to death. However, mounting evidence supports the notion that AChE inhibition alone does not explain the neurotoxic effects of sub-acute doses of OP pesticides (reviewed in Terry, 2012), particularly in the developing CNS (Slotkin and Seidler, 2008).

The translational capacity of data generated in preclinical toxicological studies is contingent upon several factors, including the appropriateness of the animal model. To date all developmental studies with sub-acute doses of CPF have been conducted on altricial species, specifically rats and mice. However, striking differences exist between their CNS development and that of humans making it difficult to extrapolate sensitive gestational periods from these rodents to humans (Byrnes et al., 2004; Dobbing and Sands, 1970, 1979). In addition, the toxicokinetics of CPF is likely to be differently influenced by pregnancy in humans and rats or mice, because, compared to humans, rats and mice have remarkably different placental structure and relatively higher levels of circulating carboxylesterases, the enzymes that metabolize and inactivate OP compounds (Carter, 2007; de Jong et al., 1993). The rodent that has relatively lower levels of circulating carboxylesterases and more closely resembles humans and primates in terms of brain development and placental structure is the guinea pig (Dobbing and Sands, 1979; Carter, 2007; de Jong et al., 1993). Therefore, the present study was designed to test the hypothesis that guinea pigs prenatally exposed to sub-acute doses of CPF develop cognitive deficits that bear resemblance to those observed in humans prenatally exposed to this pesticide.

In this study, guinea pigs were exposed *in utero* to sub-acute doses of CPF during the gestational period spanning from the time of brain growth spurt, which peaks around gestation day (GD) 50, to the time of rapid brain myelination, which peaks around GD 60 (Dobbing and Sands, 1970). When offspring reached prepubertal ages, locomotor activity and locomotor habituation, a form of non-associative memory were assessed in open fields, while spatial learning was assessed in the classic version of the Morris water maze (MWM). Data presented here support the hypothesis as they reveal that, similar to humans, guinea pigs prenatally exposed to sub-acute doses of CPF develop learning deficits, with males being more affected than females. Based on the results of this study, the guinea pig emerges as a valuable preclinical model of developmental neurotoxicity of OP pesticides.

2. Material and methods

2.1. Animal care and treatments

Pregnant Hartley guinea pigs [Crl(HA)Br; Charles River Laboratories, Wilmington, MA] were delivered to the animal facility in groups of four on presumed gestation day (GD) 33–35. There were 13 shipments. Dams were singly housed in stainless steel cages in climate-controlled rooms (21 ± 0.5 °C; 12-h light/dark cycle). Food and water were available *ad libitum*.

Starting on approximate GD 53–55 and lasting 10 consecutive days, pregnant guinea pigs received a daily subcutaneous (s.c.) injection of 25 mg/kg CPF (dissolved in peanut oil) or peanut oil. This CPF dose regimen was selected to reproduce a number of salient features associated with occupational exposures of humans to pesticides. First, the daily injections were intended to mimic the repetitive nature of these exposures (Farahat et al., 2011). Second, the s.c. route of exposure allows for a slow sustained release of the pesticide in the systemic circulation and, thereby, approximates human exposures *via* the dermal route, one of the most relevant routes of exposure to CPF (Cattani et al., 2001; Fenske et al., 2012). Third, the daily dose of CPF was selected to be below doses that induce overt signs of acute toxicity. The oral LD50 of CPF in guinea pigs is 504 mg/kg, and, in general, oral and s.c. LD50 s of OP compounds are very similar (McCollister et al., 1974). As a result, the cumulative dose of CPF used here would be well below 0.5xLD50, which is lower than the threshold for OP-induced acute toxicity (Shih and McDonough, 1997). The intention was to model a scenario in which occupational human exposure may be presumed safe.

From each delivery two mothers were injected with peanut oil and two with CPF. On rare occasions, pregnant dams died after delivery or during injections; therefore, experimental groups had offspring born from different numbers of dams (see Table 1) from different numbers of shipments (8–10). Offspring were born around GD 65–67, weaned on PND 20, and, then, housed according to their sexes in groups of 2–6 per cage. All investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.2. Measurements of cholinesterase activity

The colorimetric assay described in Fawcett et al. (2009) was used to measure AChE and butyrylcholinesterase (BuChE) activities in individual samples of red blood cells (RBCs) and plasma, respectively, collected from dams and offspring on the day of birth. Enzyme activities were also measured in extracts of different brain regions (hippocampus, thalamus, cerebral cortex, and cerebellum) harvested from offspring on the day of birth. Protein concentrations, determined using the bicinchoninic acid assay (Pierce Chemical, Rockford, IL), were used to normalize results from tissue samples. Conversion factors, 26 and 32 nmoles/AU, were used to

Table 1
Number of maternal deaths, miscarriages, litters with perinatal deaths, and offspring that died perinatally, and litter size per experimental group.

	Peanut Oil	Chlorpyrifos
Maternal deaths[a]	1/15	1/18
Miscarriages[b]	0/14	1/17
Litters with perinatal deaths[c]	7/14	5/16
Perinatal deaths[d]	14/70	10/73
Litter size[e]	5.10 ± 0.55	4.41 ± 0.42
Total number of viable female pups (range/litter)	31 (0–4)	24 (0–4)
Total number of viable male pups (range/litter)	25 (0–4)	38 (0–4)

[a] Number of pregnant guinea pigs that died during injections/total number of injected guinea pigs.
[b] Number of pregnant guinea pigs that miscarried after beginning of injections/total number of pregnant guinea pigs that survived the injections.
[c] Number of litters with offspring that died within 24 h after birth/total number of viable litters.
[d] Number of offspring that died within 24 h after birth/total number of offspring.
[e] Litter size is presented as mean ± SEM of total number of offspring per litter per group.