Available online at www.sciencedirect.com
ScienceDirect
Toxicology and Applied Pharmacology 218 (2007) 20–29
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Chlorpyrifos, chlorpyrifos-oxon, and diisopropylfluorophosphate inhibit kinesin-dependent microtubule motility

Debra A. Gearhart [a,*], Dale W. Sickles [b], Jerry J. Buccafusco [a,c], Mark A. Prendergast [d], Alvin V. Terry Jr. [a]

[a] *Department of Pharmacology and Toxicology, 1120 Fifteenth Street, Medical College of Georgia, Augusta, GA 30912-2300, USA*
[b] *Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912, USA*
[c] *Veterans Administration Medical Center, Augusta, GA 30904, USA*
[d] *Department of Psychology, University of Kentucky Lexington, KY 40506, USA*

Received 25 April 2006; revised 1 October 2006; accepted 11 October 2006
Available online 17 October 2006

Abstract

Diisopropylfluorophosphate, originally developed as a chemical warfare agent, is structurally similar to nerve agents, and chlorpyrifos has extensive worldwide use as an agricultural pesticide. While inhibition of cholinesterases underlies the acute toxicity of these organophosphates, we previously reported impaired axonal transport in the sciatic nerves from rats treated chronically with subthreshold doses of chlorpyrifos. Those data indicate that chlorpyrifos (and/or its active metabolite, chlorpyrifos-oxon) might directly affect the function of kinesin and/or microtubules—the principal proteins that mediate anterograde axonal transport. The current report describes *in vitro* assays to assess the concentration-dependent effects of chlorpyrifos (0–10 μM), chlorpyrifos-oxon (0–10 μM), and diisopropylfluorophosphate (0–0.59 nM) on kinesin-dependent microtubule motility. Preincubating bovine brain microtubules with the organophosphates did not alter kinesin-mediated microtubule motility. In contrast, preincubation of bovine brain kinesin with diisopropylfluorophosphate, chlorpyrifos, or chlorpyrifos-oxon produced a concentration-dependent increase in the number of locomoting microtubules that detached from the kinesin-coated glass cover slip. Our data suggest that the organophosphates–chlorpyrifos-oxon, chlorpyrifos, and diisopropylfluorophosphate–directly affect kinesin, thereby disrupting kinesin-dependent transport on microtubules. Kinesin-dependent movement of vesicles, organelles, and other cellular components along microtubules is fundamental to the organization of all eukaryotic cells, especially in neurons where organelles and proteins synthesized in the cell body must move down long axons to pre-synaptic sites in nerve terminals. We postulate that disruption of kinesin-dependent intracellular transport could account for some of the long-term effects of organophosphates on the peripheral and central nervous system.

Keywords: Diisopropylfluorophosphate; DFP; Chlorpyrifos; Chlorpyrifos-oxon; Organophosphorus; Organophosphates; Kinesin; Kinesin superfamily (KIF); Tubulin; Microtubules; Axonal transport; Pesticides; Microtubule motility; Cholinesterase

Introduction

One aspect of our research effort is to discover the mechanisms that account for the delayed, long-term cognitive impairment that follows chronic "subthreshold exposure" to organophosphates, such as chlorpyrifos (Terry, 2003). We define "subthreshold exposure" as dosing regimens of organophosphates that do not produce overt signs of cholinergic toxicity—such as muscle fasciculations, flaccid paralysis (including respiratory muscles), seizures, diarrhea, urination, meiosis, salivation, and lacrimation (see reviews by: Rusyniak and Nanagas, 2004; Sungurtekin et al., 2006). Prolonged exposure to either diisopropylfluorophosphate or chlorpyrifos appears to be associated with cognitive deficits (see reviews by: Overstreet, 2000; Kamel and Hoppin, 2004), and both compounds may produce neuropathies (reviewed in Lotti and Moretto, 2005) and neurotoxicity (reviewed by Jamal et al., 2002) depending on the dose or length of exposure (reviewed in Costa, 2006). We are evaluating diisopropylfluorophosphate (an alkylphosphate) and chlorpyrifos (a pyridyl phosphorothioate) because these organophosphates: (1) are structurally different

* Corresponding author. Fax: +1 706 721 2347.
E-mail address: dgearhar@mcg.edu (D.A. Gearhart).

0041-008X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2006.10.008

(see Fig. 1) and (2) have distinct binding and inhibition profiles with respect to acetylcholinesterase and neurotoxic esterase (see reviews by: Reiner, 2001; Kropp and Richardson, 2003). We do not intend to group together diisopropylfluorophosphate with chlorpyrifos with respect to their toxicity. However, there is accumulating evidence that chronic exposure of rats to low doses of chlorpyrifos produces neurobehavioral (cognitive) and neurochemical deficits (Bushnell et al., 1994; Canadas et al., 2005; Moser et al., 2005).

Diisopropylfluorophosphate, originally developed as a chemical warfare agent, is structurally similar to nerve agents (e.g., sarin and soman), and chlorpyrifos is extensively used worldwide as an agricultural pesticide (reviewed by Donaldson et al., 2002). The U.S. Environmental Protection agency recently banned the use of chlorpyrifos in U.S. homes (EPA, 2002). However, chlorpyrifos is still extensively used as an agricultural pesticide and in controlling pests in and around commercial buildings (Lemus and Abdelghani, 2000). Evidence of the widespread use of chlorpyrifos is exemplified by the report of detectable 3,5,6-trichloro-2-pyridinol (TCP), a metabolite of chlorpyrifos, in the urine of 96% of ~2000 samples collected from individuals (ages 2–59 years) living in the U.S. (Barr et al., 2005).

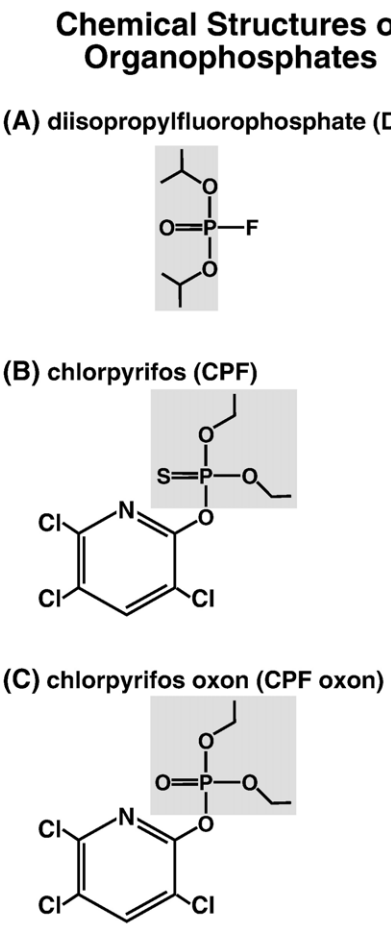

Fig. 1. The chemical structures of the organophosphates–(A) diisopropylfluorophosphate, (B) chlorpyrifos, (C) chlorpyrifos-oxon (reactive metabolite of chlorpyrifos)–tested in kinesin-dependent microtubule motility assays. The part of the organophosphate shaded in gray shows the portion of the structure that is covalently bound to cholinesterases (and probably other proteins) after the serine hydroxyl group (in the active site of cholinesterase) effects a nucleophilic attack on the phosphorous atom of the organophosphate.

The acute toxicity of chlorpyrifos, chlorpyrifos-oxon (the reactive metabolite of chlorpyrifos), and diisopropylfluorophosphate is due to inhibition cholinesterase enzymes, including acetylcholinesterase (Amitai et al., 1998). At cholinergic synapses, acetylcholinesterase activity rapidly hydrolyzes acetylcholine to choline and acetate. Therefore, acetylcholinesterase inhibition causes excessive accumulation of synaptic acetylcholine, which continually stimulates cholinergic receptors in the central nervous system, autonomic nervous system, and neuromuscular junction (reviewed by Pope et al., 2005). *In vivo*, chlorpyrifos toxicity is primarily due to P450 monooxygenase-mediated metabolism of chlorpyrifos to chlorpyrifos-oxon, which is a more potent acetylcholinesterase inhibitor than chlorpyrifos (Tang et al., 2001).

Due to their highly reactive nature, it is conceivable that organophosphates might alter the function of other enzymes and/or structural proteins (see reviews by: Casida and Quistad, 2005; Lopachin and Decaprio, 2005). Interactions of organophosphates with non-cholinesterase targets may contribute to the more delayed and persistent effects of organophosphates observed following chronic exposure to subthreshold doses of organophosphates (see reviews by: Lotti and Moretto, 2005; Costa, 2006). For example, we reported impaired anterograde axonal transport in sciatic nerves that were isolated from rats exposed to repeated subthreshold doses of chlorpyrifos (Terry et al., 2003) or diisopropylfluorophosphate [unpublished data]. Briefly, subcutaneous doses of 2.5, 10, 18, or 25 mg/kg/day chlorpyrifos for 14 days inhibited plasma cholinesterase activity by 30–60%, without eliciting overt signs of organophosphate toxicity. Notably, the 18 and 25 mg/kg doses impaired water maze performance and swim speeds for up to 5 days after the last dose. In addition, 6 days after discontinuing the 14-day 10.0, 18.0, and 25.0 mg/kg chlorpyrifos dosing regimens, there was impaired anterograde (50–75% of control) and retrograde (55–80% of control) axonal transport (measured *ex vivo* in sciatic nerves from study animals). Surprisingly, measurable deficits in axonal transport (85–90% of control) persisted for 20 days after the last dose in chlorpyrifos-treated rats (25.0 mg/kg × 14 days)—even though water maze performance and grip strength appeared normal. Other (primarily indirect) observations appear to support our finding that chlorpyrifos affects axonal transport; specifically, organophosphorus agents that produce delayed neurotoxicity (at high doses) cause accumulations of tubulovesicular profiles within axons prior to axonal degeneration (reviewed by Abou-Donia and Lapadula, 1990), a pathology that is consistent with stagnation of membrane traffic (Chretien et al., 1981; Souyri et al., 1981). We postulate that chlorpyrifos might also impair axonal transport in the central nervous system (CNS) and that impaired axonal transport in CNS cholinergic pathways could play a role in the cognitive dysfunction observed after chlorpyrifos exposure. Collectively, these data indicate that chlorpyrifos, chlorpyrifos-oxon, and/or diisopropylfluorophosphate could directly affect the function of the principal proteins–kinesin and microtubules (polymerized α