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Neurotoxicity in murine striatal dopaminergic pathways following co-application of permethrin, chlorpyrifos, and MPTP

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Abstract

The neurotoxic action of permethrin and chlorpyrifos on striatal dopaminergic pathways was investigated in C57BL/6 mice. Technical permethrin (50/50 ratio of *cis* and *trans* isomers, 200 mg/kg) and/or chlorpyrifos (75 mg/kg) were administered three times over a two-week period, with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 20 mg/kg) given on day one. Alterations in expression of α-synuclein, dopamine transporter (DAT), and tyrosine hydroxylase (TH) were analyzed at 1 or 28 days post-treatment. MPTP alone produced a long-lasting lesion in striatal dopaminergic pathways, with a depression of TH and DAT protein at both post-treatment times. Chlorpyrifos or permethrin alone had no effect on TH or DAT expression levels. No greater effect on protein expression was observed in mice treated with both MPTP and insecticides at 1 day post-treatment. However, by day 28 a significant reduction ($p < 0.05$) of TH and DAT was observed in the mice treated with MPTP, permethrin, and chlorpyrifos, compared with the mice given MPTP alone. Significant alteration ($p < 0.05$) of α-synuclein expression by MPTP (45% decrease) and permethrin (20% increase) occurred at 1 day post-treatment, but reverted to control levels by day 28. Parallel experiments with pure *cis* or *trans* isomers of permethrin (100 mg/kg), showed that each isomer caused about half the up-regulation of α-synuclein. These findings demonstrate that the co-application of pyrethroid or organophosphorus insecticides enhance the neurotoxicity of MPTP in C57BL/6 mice, and that a slowly developing neurotoxicity may occur after termination of high-dose exposure.

Keywords: Parkinson's disease; Insecticide; Striatum; Dopamine transporter; Tyrosine hydroxylase; α-Synuclein; Synaptophysin

1. Introduction

PM and CPF[2] are two widely used insecticides in the pyrethroid and organophosphorus compound classes. PM and CPF exposure may be contributory to Gulf War syndrome, since approximately 30,000 veterans complained of neurological symptoms after exposure to multiple chemicals, in particular PB, DEET, PM, and CPF [1–4]. Animal experiments indicated that relatively high doses of PB, DEET, PM, or CPF alone caused minimal neurotoxicity, while co-exposure to the same doses of these compounds significantly increased the severity of motor deficits [1]. Thus, mixed exposures to neurotoxicants may have unforeseen effects.

Epidemiological studies [5] have implicated organophosphorus insecticide exposure as a possible contributory factor in PD. Moreover, previous studies revealed that PM and CPF affect the nigro-striatal system, the primary brain pathway lesioned in PD [6]. PM enhanced dopamine uptake [7] and increased DAT expression [8] at the dose of 1.5 mg/kg to C57BL/6 mice. Similar effects of other pyrethroid insecticides on striatal dopaminergic neurochemistry, such as deltamethrin, has also been documented [9,10]. At high doses, both PM (200 mg/kg) and CPF (100 mg/kg) significantly reduce maximal [^{3}H]dopamine uptake and caused a decrease

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[2] *Abbreviations used:* CPF, chlorpyrifos; DAT, dopamine transporter; DEET, *N*,*N*-diethyltoluamide; DOPAC, dihydroxyphenylacetic acid; DTT, dithiothreitol; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTG, methoxytriglycol; PB, pyridostigmine bromide; PD, Parkinson's disease; PM, permethrin; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

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in locomotor activity [7]. In addition, PM treatment up-regulated α-synuclein [8], an important component of Lewy bodies, proteinaceous tangles found in PD [11]. Taken together, these findings suggest that dopaminergic neurotransmission is significantly affected by exposure to pyrethroid and organophosphorus insecticides.

The present study is focused on the neurotoxic actions of PM (200 mg/kg) and CPF (75 mg/kg) alone or in combination, and their interactions with the well-established Parkinsonian neurotoxin, MPTP. This approach provides an opportunity to assess the ability of insecticides to intensify the development of PD, as modeled by exposure to MPTP. Western blot analyses were conducted to evaluate the alteration of biomarkers specific to dopaminergic pathways in striatum, including the expression of DAT and TH proteins. Reduction of DAT and TH indicates dopaminergic nerve terminal injury, because DAT and TH are mainly expressed in dopaminergic terminals and great reductions in DAT and TH expression occurs in the brains of MPTP-lesioned mice [12,13] or human Parkinsonian patients [14]. Moreover, previous studies (e.g., Tillerson et al. [13]) showed that Western blot measures of DAT and TH expression are appropriate and selective markers for striatal dopaminergic neurotoxicity. We also evaluated expression of α-synuclein, as well as synaptophysin; the latter an abundant synaptic protein widely expressed in neurons [15], as a check for specificity of effect.

2. Materials and methods

2.1. Chemicals

Technical PM (a 50/50 mixture of 1-*R*,*S*-*cis* and 1-*R*,*S*-*trans* isomers, Fig. 1) was purchased from Sigma–Aldrich GMBH. CPF (99%) and the resolved *cis* (99%) and *trans* (94%) isomers of PM were obtained from ChemService (West Chester, PA). The MPTP used originated from Research Biochemicals International, Natick, MA. Buffer components were purchased from Fisher Chemicals, Fair Lawn, NJ. Bio-Rad (Hercules, CA) was the commercial source for 30% acrylamide, ammonium persulfate, SDS, and TEMED. Rat monoclonal anti-DAT, mouse monoclonal anti-TH, and anti-synaptophysin primary antibodies were purchased from Chemicon International (Temecula, CA). Mouse monoclonal anti-α-synuclein primary antibody was obtained from Biodesign International (Saco, ME). The secondary peroxidase-linked antibody was from Sigma Chemical (St. Louis, MO). ECL Western blotting detection system RPN 2108 and ECL Hyperfilm were purchased from Amersham-Pharmacia Biotech (Buckinghamshire, UK).

2.2. Animals and treatments

C57BL/6 retired breeder male mice (Harlan–Sprague–Dawley, Dublin, VA) were used for all the experiments, which were approved by the Virginia Tech Animal Care and Use Committee. Mice aged 7–9 months, weighing from 36 to 42 g were used because this age range gives a consistent dopamine depletion following MPTP treatment [16]. Mice were randomly assigned to treatment groups according to weight, with typically five mice in each group. The mean weight of all treatment groups was not significantly different. High, but sublethal doses of PM (200 mg/kg) and CPF (75 mg/kg) were selected in the present study based on our previous studies of these two insecticides in C57BL/6 mice [7]. A single dose of 20 mg/kg MPTP was chosen because this dose caused approximately

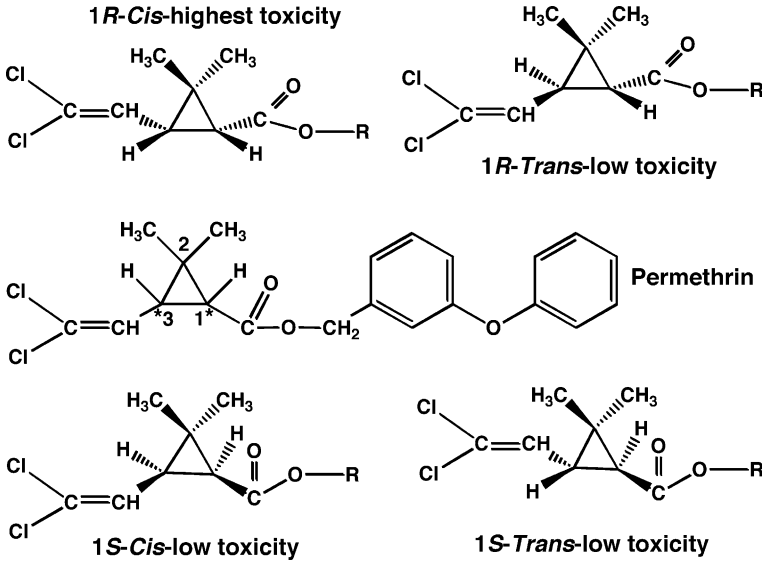

Fig. 1. Chemical structures of the PM isomers discussed in the text. In the middle generic structure, chiral carbon atoms 1 and 3 in the cyclopropane ring are indicated by asterisks. Consensus pyrethroid nomenclature defines the *R* or *S* stereochemistry at carbon 1, with *cis* or *trans* designations defined by projections relative to the cyclopropane ring, so as to consistently define biologically active conformations, as opposed to formal stereoisomeric nomenclature, as described by [34]. Relative mammalian toxicities for PM isomers, are as given by Soderlund et al. [33].