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Effect of *in vivo* nicotine exposure on chlorpyrifos pharmacokinetics and pharmacodynamics in rats

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ABSTRACT

Routine use of tobacco products may modify physiological and metabolic functions, including drug metabolizing enzymes, which may impact the pharmacokinetics of environmental contaminants. Chlorpyrifos is an organophosphorus (OP) insecticide that is bioactivated to chlorpyrifos-oxon, and manifests its neurotoxicity by inhibiting acetylcholinesterase (AChE). The objective of this study was to evaluate the impact of repeated nicotine exposure on the pharmacokinetics of chlorpyrifos (CPF) and its major metabolite, 3,5,6-trichloro-2-pyridinol (TCPy) in blood and urine and also to determine the impact on cholinesterase (ChE) activity in plasma and brain. Animals were exposed to 7-daily doses of either 1 mg nicotine/kg or saline, and to either a single oral dose of 35 mg CPF/kg or a repeated dose of 5 mg CPF/kg/day for 7 days. Groups of rats were then sacrificed at multiple time-points after receiving the last dose of CPF. Repeated nicotine and CPF exposures resulted in enhanced metabolism of CPF to TCPy, as evidenced by increases in the measured TCPy peak concentration and AUC in blood. However, there was no significant difference in the amount of TCPy (free or total) excreted in the urine within the first 24-h post last dose. The extent of brain acetylcholinesterase (AChE) inhibition was reduced due to nicotine co-exposure consistent with an increase in CYP450-mediated dearylation (detoxification) versus desulfuration. It was of interest to note that the impact of nicotine co-exposure was experimentally observed only after repeated CPF doses. A physiologically based pharmacokinetic model for CPF was used to simulate the effect of increasing the dearylation V_{max} based upon previously conducted *in vitro* metabolism studies. Predicted CPF-oxon concentrations in blood and brain were lower following the expected V_{max} increase in nicotine treated groups. These model results were consistent with the experimental data. The current study demonstrated that repeated nicotine exposure could alter CPF metabolism *in vivo*, resulting in altered brain AChE inhibition.

1. Introduction

Chlorpyrifos (CPF) is a widely utilized organophosphorus pesticide (OP), which has been extensively studied due to its potential neurotoxicity [1–3]. Although CPF is no longer registered for residential use in the US [4], there is still a high potential for human exposure, since it is widely utilized in agricultural applications [5]. Workers who are directly involved in agricultural operations may constitute a population who could face different risk from exposure to mixtures of chemicals [6]. The long-term goal of this research is to better understand the implications of the use of tobacco products on agricultural workers who may also be exposed to a range of insecticides. The primary mode of action for the toxicity of OPs, including CPF, involves the inhibition of acetylcholinesterase (AChE) activity [3,7]. Cholinesterases (ChE) are a broad class of ubiquitous enzymes that are present in many tissues including brain and muscle. CPF itself is a weak inhibitor of AChE, but it is bioactivated by cytochromes P450 (CYP450s) to the more potent inhibitor, chlorpyrifos-oxon (CPF-oxon). The inhibition of AChE by CPF-oxon results in an excessive accumulation of acetylcholine within the cholinergic synapse and neuromuscular junctions, thereby leading to a wide range of neurotoxic responses [8].

Chlorpyrifos is metabolized by CYP450s to several major metabolites, including CPF-oxon and 3,5,6-trichloro-2-pyridinol (TCPy) [9]. Once CPF is metabolized to TCPy, this metabolite can be directly excreted in the urine or undergo further conjugation prior to urinary excretion [10]. In addition, the conversion of CPF-oxon to TCPy is mediated by several esterases. While A-esterases, such as paroxonase-1 (PON-1), convert oxon to TCPy and are not inhibited

Abbreviations: OP, organophosphorus; CPF, chlorpyrifos; CPF-oxon, chlorpyrifos-oxon; TCPy, 3,5,6-trichloro-2-pyridinol; AChE, acetylcholinesterase; ChE, cholinesterase; CYP450, cytochrome P450; AUC, area-under-the-curve; PON-1, paroxonase-1; sc, subcutaneous; PBPK/PD, physiologically based pharmacokinetic and pharmacodynamic.

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by the oxon, whereas B-esterases, including AChE, BuChE and carboxylesterase (CaE), have a serine residue at the active site and are inhibited in the process. Since CYP450 plays a central role in both the activation and detoxification of CPF, changes in enzyme activity may have a profound impact on CPF dosimetry and the extent of esterase inhibition.

A number of studies have demonstrated the inductive effects of nicotine (the major component of tobacco products) on CYP450 metabolism both *in vivo* and *in vitro* [11,12]. Additionally, the influence of nicotine on physiological and pharmacodynamic processes, such as increases in heart rate, blood pressure, and energy expenditure, are well documented [13,14]. It has also been shown that prolonged exposure to even low concentrations of nicotine can cause desensitization of nicotinic receptors in the brain [15]. These diverse effects of nicotine exposure could subsequently modulate key internal pharmacokinetic and pharmacodynamic processes and alter the adverse effect due to exposures to OPs. There have been a few reports showing the combined effects due to co-exposures to CPF and nicotine [16,17], including one by Qiao et al. which suggested the combination of nicotine and CPF may result in increased thiobarbituric-acid-reactive species (TBARS) in developing rats [17].

We recently reported that repeated *in vivo* nicotine pretreatments impacted *in vitro* CPF metabolism in rat liver microsomes [18]. The influence of repeated pre-exposure to nicotine on CPF metabolism did not affect bioactivation to the CPF-oxon, but did result in increased dearylation of CPF to TCPy (1.9-fold induction of TCPy V_{max}). The current study was conducted to quantitatively assess potential pharmacokinetic changes in CPF and TCPy in blood and the pharmacodynamic ChE responses to CPF exposures in key tissues following pre- and co-exposures to nicotine. Levels of ChE inhibition in plasma and brain were measured to characterize changes in pharmacodynamic dose–response and also provide some qualitative perspective on the target tissue dose of CPF-oxon, which is too labile to measure analytically.

2. Materials and methods

2.1. Chemicals

Chlorpyrifos (CAS: 2921-88-2, 99% pure) and 3,5,6-trichloro-2-pyridinol (CAS: 6515-38-4, 99% pure] were kindly provided by Dow AgroSciences (Indianapolis, IN). Nicotine [as (−)-1-methyl-2-(3-pyridyl)pyrrolidine-(+)-bitartrate salt], the derivatizing agent, N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA), along with Ellman reagents, 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB] and acetylthiocholine chloride (ATC) were purchased from Sigma Chemical Company (St Louis, MO). The remaining chemicals and other solvents were reagent grade or better and were purchased from Sigma Chemical Company.

2.2. Animals

Adult male Sprague–Dawley rats (200–225 g, ca. 7 weeks old) were purchased from Charles River Laboratories, Inc. (Raleigh, NC). Prior to use, animals were randomly assigned to naïve and treatment groups and all animals were housed in solid-bottom cages with hardwood chips, and acclimated for 1 week in a humidity- and temperature-controlled room with a 12-h light/dark cycle. Rodent feed (PMI Certified Rodent Diet # 5002) and water were provided ad libitum. All procedures described in the present study were conducted in accordance with the guidelines for the care and use of laboratory animals in the NIH/NRC Guide and Use of Laboratory Animals, and were approved by the Institutional Animal and Care Use Committee (IACUC) of Battelle, Pacific Northwest Division.

2.3. Dose selection

There were three goals in selection of CPF dose, first that major metabolites could be quantified in plasma, second that cholinesterase inhibition could be measured in blood and brain, and finally so that subtle differences in cholinesterase inhibition could be detected. The selection of CPF doses employed in this study were based upon predictions from CPF PBPK model simulations in addition to previous experience and examination of ample literature regarding CPF extensively reviewed by Eaton et al. [7]. The repeated doses of 5 mg CPF/kg/day were predicted to elicit ∼75% cholinesterase inhibition in plasma and ∼50% inhibition in brain after 7 days of treatment, while they were not expected to result in extreme systemic cholinergic symptoms, based upon previous studies with repeated CPF treatments and dietary exposure in rats [19,20]. The oral single dose of 35 mg CPF/kg, equivalent to the total repeated dose, was expected to produce mild cholinergic symptoms, resulting in the similar levels of ChE inhibition in blood and brain with those from repeated 5 mg CPF/kg/day for 7 days, according to CPF PBPK model simulations (data not shown).

The dose and route for nicotine exposures (1 mg/kg/day for 7 days, sc) were based on nicotine CYP450 induction studies [11,12,21], due to the rapid metabolism of nicotine in rodent, these relatively high daily doses of nicotine are necessary to achieve blood nicotine concentrations comparable to those seen in smokers [22]. Doses of 1 mg nicotine/kg/day in rats were shown to produce similar CNS effects of nicotine found in smokers [23], and the pharmacological impacts of these nicotine doses compared to cigarette smoking have been extensively reviewed by Benowitz (1996, 2009) and Tricker (2006).

2.4. Animal treatment

Animals were randomly assigned to either the naïve group ($n = 4$) or CPF-treatment groups ($n = 4$–5 per group) with different post-dosing time points (1, 4, 8, 12 or 24 h) for sacrifice (Table 1). These CPF-treated groups included I) [CPF Repeat Dose]: subcutaneous (sc) saline + 5 mg CPF/kg (in corn oil, po) daily for 7 days; II) [Nicotine + CPF Repeat Dose]: 1 mg nicotine/kg/day + 5 mg CPF/kg/day (in corn oil, po) for 7 days; III) [CPF Single Dose]: saline for 7 days, and a single 35 mg CPF/kg (in corn oil, po) on the 7th day; and IV) [Nicotine + CPF Single Dose]: 1 mg nicotine/kg/day, for 7 days and a single dose of 35 mg CPF/kg (in corn oil, po) on 7th day. Rats were dosed with saline or 1 mg nicotine/kg (in sterile saline) subcutaneously (sc) at the nape in a dose volume of 1 ml/kg body weight, which demonstrated metabolic induction of CYP450s [18,21]. Oral CPF was dosed in corn oil (5 ml/kg b.w.) within 5 min of nicotine or saline dosing. Animals in the 24-h groups (both saline and nicotine treatments) were individually housed in plastic metabolism cages (Thermo Fisher Scientific, Rochester, NY) for at least 48 hr prior to the last CPF dose for acclimation. Urine was collected continuously with 12-h intervals post CPF treatment. Rats were euthanized by CO_2 asphyxiation at the specified time-points post-last CPF dosing (Table 1), and blood was collected via cardiac puncture into heparinized syringes. Plasma from each animal was prepared by centrifugation of blood at 1100 × *g* for 10 min. Immediately after blood collection, the brain was removed, and dry blotted, homogenized with nine volumes of ice-cold 0.1 M phosphate buffer (pH 7.4), all samples were stored at −80 °C until analyzed. The same volume of blood/urine from each individual animal and blank matrices from the naive animals were treated with 250 μL of NaCl-saturated 3N HCl. For analysis of conjugated TCPy in urine sample, urine aliquots were hydrolyzed by adding concentrated HCl and heated at 80° C for 1 h prior to extraction. Blood/urine samples were extracted 3× with 0.75 ml ethyl acetate, vortexed in a shaking incubator for 10 min, centrifuged to separate layers at 1100 × *g*