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Pharmacokinetics and effects on serum cholinesterase activities of organophosphorus pesticides acephate and chlorpyrifos in chimeric mice transplanted with human hepatocytes

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ABSTRACT

Organophosphorus pesticides acephate and chlorpyrifos in foods have potential to impact human health. The aim of the current study was to investigate the pharmacokinetics of acephate and chlorpyrifos orally administered at lowest-observed-adverse-effect-level doses in chimeric mice transplanted with human hepatocytes. Absorbed acephate and its metabolite methamidophos were detected in serum from wild type mice and chimeric mice orally administered 150 mg/kg. Approximately 70% inhibition of cholinesterase was evident in plasma of chimeric mice with humanized liver (which have higher serum cholinesterase activities than wild type mice) 1 day after oral administrations of acephate. Adjusted animal biomonitoring equivalents from chimeric mice studies were scaled to human biomonitoring equivalents using known species allometric scaling factors and *in vitro* metabolic clearance data with a simple physiologically based pharmacokinetic (PBPK) model. Estimated plasma concentrations of acephate and chlorpyrifos in humans were consistent with reported concentrations. Acephate cleared similarly in humans and chimeric mice but accidental/incidental overdose levels of chlorpyrifos cleared (dependent on liver metabolism) more slowly from plasma in humans than it did in mice. The data presented here illustrate how chimeric mice transplanted with human hepatocytes in combination with a simple PBPK model can assist evaluations of toxicological potential of organophosphorus pesticides.

1. Introduction

Biomonitoring techniques for determining internal doses of chemicals have become valuable tools for quantitatively evaluating human exposure from environmental and/or accidental/incidental sources. The organophosphorus pesticide acephate (Fig. 1) is one of the most widely used pesticides in agriculture. Acephate is bioactivated to the more potent methamidophos, which acts as an acetylcholinesterase inhibitor (Mahajna et al., 1997). There are few case reports of incidental human ingestion of acephate (Chang et al., 2009; Joint FAO/WHO Meeting, 2003). An acetylcholinesterase biosensor has been developed to detect organophosphorus pesticides acephate and malathion in food samples (Raghu et al., 2014).

Chlorpyrifos (Fig. 1), another organophosphorus pesticide, is reportedly extensively activated to chlorpyrifos-oxon and deactivated to trichloropyridinol in rats (Smith et al., 2011; Tang et al., 2001). A benchmark dose analysis (Marty et al., 2012) and meta-analysis (Reiss et al., 2012) have been published for chlorpyrifos and recommended equivalent benchmark doses of 1.7 mg/kg/day for children and adults after a single acute dose and 0.67 mg/kg/day after repeated doses. Physiologically based pharmacokinetic (PBPK) models and/or pharmacodynamic models have been reported for chlorpyrifos, including models for rats and humans (Timchalk et al., 2002), chemical mixtures (Timchalk and Poet, 2008), gestational exposure (Lowe et al., 2009), specific age groups (3 and 30 years) linked to dietary exposure (Hinderliter et al., 2011), predictions of two age groups (1 and 19 years) (Foxenberg et al., 2011), and human life stage (Smith et al., 2014). Dietary exposure levels of chlorpyrifos have been studied extensively.

The complicated multiple compartments and equations found in traditional PBPK modeling cause severe difficulties for many regulatory and industrial researchers when applying the model. Recently developed TK-NOG mice transplanted with human liver

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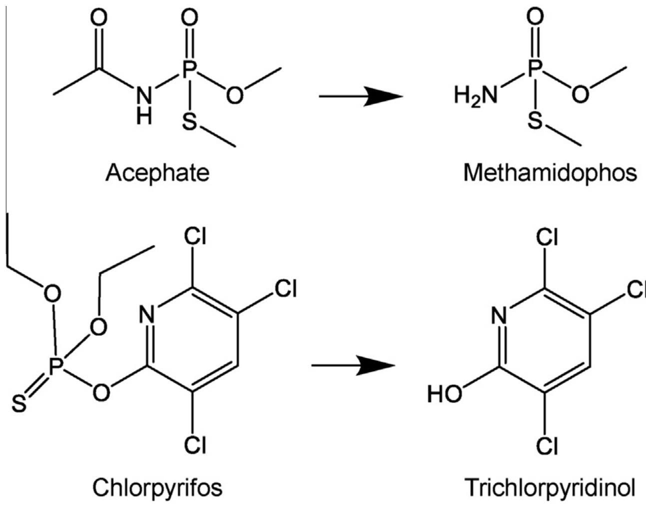

Fig. 1. Chemical structures of acephate and chlorpyrifos and their metabolites.

cells can survive without ongoing drug treatment (Hasegawa et al., 2011; Yamazaki et al., 2013), something that was not possible in previous liver reconstruction animal models. Consequently, with transplanted TK-NOG mice it is now possible to evaluate drug interactions (Yamazaki et al., 2013) and estimates obtained from simplified human PBPK modeling by comparing the predicted values with *in vivo* experimental results from mice with humanized liver. Applying this approach, we reported simplified PBPK models (Tsukada et al., 2013; Yamashita et al., 2014) for estimating the internal doses of melengestrol acetate and molinate and their key metabolites based on data from chimeric mice transplanted with human hepatocytes to better understand and evaluate toxicological studies in humans.

In the present study, the pharmacokinetics of acephate and chlorpyrifos in chimeric mice transplanted with human hepatocytes were investigated. Our observations showed that human hepatocytes were able to elevate serum cholinesterase activities in chimeric mice. A simplified PBPK model was able to estimate human plasma concentrations of acephate and chlorpyrifos after accidental ingestion and was capable of both forward and reverse dosimetry.

2. Materials and methods

2.1. Chemicals, animals, and enzyme preparations

Acephate (*O*,*S*-dimethyl acetylphosphoramidothioate), methamidophos (*O*,*S*-dimethyl phosphoramidothioate), chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), and trichloropyridinol (3,5,6-trichloro-2-pyridinol) were purchased from Wako Pure Chemicals, Tokyo, Japan. Microsomes from pooled human livers (H150) were obtained from Corning (Woburn, MA, USA). Liver microsomes from 7-week-old male Sprague-Dawley rats and mice were prepared as described previously (Tsukada et al., 2013; Yamashita et al., 2014). Recently developed TK-NOG mice (Hasegawa et al., 2011; Higuchi et al., 2014; Yamazaki et al., 2012) are severely immunodeficient NOG (non-obese diabetes-severe combined immunodeficiency interleukin-2 receptor gamma chain-deficient) mice treated to express herpes simplex virus type 1 thymidine kinase (HSVtk) in their livers. Liver cells expressing HSVtk are ablated by a non-toxic dose of ganciclovir, and human liver cells can then be transplanted without the need for ongoing drug treatment. Wild type mice (TK-NOG mice with no transplanted human hepatocytes) and humanized TK-NOG mice (~20–30 g body weight) (Hasegawa et al., 2011) were used in this study. In the chimeric mice, more than 70% of liver cells were estimated to have been replaced with human hepatocytes, as judged by measurements of human albumin concentrations in plasma (Hasegawa et al., 2011; Yamazaki et al., 2012). Hereafter, the terms "mouse" or "mice" refer to wild type TK-NOG mice. The use of animals for this study was approved by the Ethics Committees of the Central Institute for Experimental Animals and Showa Pharmaceutical University. Plasma samples were collected 0.5, 1, 2, 4, 7, and 24 h after single oral doses of acephate (150 mg/kg) or chlorpyrifos (30 mg/kg), these doses being the lowest-observed-adverse-effect levels (Joint FAO/WHO Meeting, 2003). Accumulated urine samples (0–7 h) were also collected. After treatment of the plasma or urine samples (50 μL) from individual mice with methanol (100 μL) for deproteinization, protein concentrations were estimated by using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA). Other reagents used in this study were obtained from sources described previously or were of the highest quality commercially available (Tsukada et al., 2013; Yamashita et al., 2014).

2.2. In vivo and in vitro metabolic studies of acephate and chlorpyrifos

The levels of substrates and their metabolites in plasma samples and in *in vitro* incubation mixtures were determined by liquid chromatography (LC). The levels of acephate and its metabolite in the plasma samples were determined by an LC/tandem mass spectrometry (MS) system as described (Montesano et al., 2007) with modifications. An LCQ Duo mass analyzer (ThermoFisher Scientific, Yokohama, Japan) equipped with Xcalibur software was operated in electrospray positive ionization mode and was directly coupled to an Agilent 1100 system (Agilent Technology, Tokyo, Japan) equipped with an octadecylsilane (C_{18}) column (XBridge, 3.5 μm, 2.1 mm × 150 mm, Waters, Tokyo, Japan). LC conditions were as follows: buffer A contained 5 mM ammonium acetate in methanol and buffer B contained 5 mM ammonium acetate. The following gradient program was used at a flow rate of 0.20 mL/min: 0–6 min, linear gradient from 20% A to 45% A (v/v); 6–7 min, linear gradient from 45% A to 95% A (v/v); 7–12 min, hold at 95% A; 12–13 min, linear gradient from 95% A to 20% A (v/v); 13–19 min, hold at 20% A. The temperature of the column was maintained at 35 °C. Samples (5 μL) were infused with an auto-sampler and MS analyses were performed. To tune the mass spectrometer, solutions of acephate and methamidophos (10 ppm in a mobile phase) were infused into the ion source, and the cone voltage was optimized to maximize the intensity of the precursor ions for the most abundant acephate and methamidophos product ions (*m/z* 184.0 and 142.0, respectively). Acephate and methamidophos were quantified using the *m/z* 184.0 → 142.9 transition of acephate and the *m/z* 142.0 → 112.0 transition of methamidophos. Typical tuning conditions for acephate and methamidophos were as follows: electrospray capillary voltage, 4.5 kV; sample cone voltage, 13 V; collision energy, 33 and 28 eV, respectively; capillary temperature 225 °C; and sheath gas pressure 35 and auxiliary gas pressure 20 (arbitrary units) of helium.

Another LC system, consisting of a pump and multi-wavelength UV detector (Shimadzu, Kyoto, Japan) with an analytical C_{18} reversed-phase column (5 μm, 4.6 × 250 mm, Mightysil RP-18 GP, Kanto Chemicals, Tokyo, Japan), was used for acephate in the following incubation mixtures: solution A contained 5 mM ammonium acetate in methanol and solution B contained 5 mM ammonium acetate in water. The following gradient program was used at a flow rate of 1.0 mL/min: 0–1 min, hold at 5% A; 1–6 min, linear gradient from 5% A to 45% A (v/v); 6–7 min, linear gradient from 45% A to 95% A (v/v); 7–12 min, hold at 95% A; 12–13 min, linear gradient from 95% A to 5% A (v/v); 13–19 min, hold at 5% A.

LC conditions for chlorpyrifos in the plasma samples and *in vitro* incubation mixtures were as follows. Solution A contained CH_3CN

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