Journal of Pharmacological and Toxicological Methods 70 (2014) 106–111

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Measurement of plasma protein and lipoprotein binding of pyrethroids

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ARTICLE INFO

Article history:
Received 21 March 2014
Accepted 2 June 2014
Available online 11 June 2014

Keywords:
Binding
Cyclosporine binding
Deltamethrin
Diazepam binding
Lipoproteins
Method
Permethrin
Plasma proteins
Pyrethroid
Solvent extraction

ABSTRACT

Introduction: A simple, reliable procedure was developed to measure binding of pyrethroid insecticides to total proteins and lipoproteins of rat and human plasma. **Methods:** The extent of binding of ^{14}C-labeled deltamethrin (DLM), cis-permethrin (CIS) and trans-permethrin (TRANS) was quantified by a 3-step organic solvent extraction technique. Rat and human plasma samples, containing NaF to inhibit esterases, were spiked with a range of concentrations of each radiolabeled pyrethroid. Protein binding reached equilibrium within ~1 h of incubation at 37 °C. The samples were extracted in turn with: isooctane to collect the unbound fraction; 2-octanol to extract the lipoprotein-bound fraction; and acetonitrile to obtain the protein-bound fraction. **Results:** Absolute recoveries of DLM, CIS and TRANS ranged from 86 to 95%. Adherence of these very lipophilic chemicals to glass and plastic was minimized by using silanized glass vials and LoBind® plastic pipettes. The method's ability to distinguish lipoprotein from protein binding was confirmed by experiments with diazepam and cyclosporine, drugs that bind selectively to albumin and lipoproteins, respectively. **Discussion:** This procedure was effectively utilized for studies of the species-dependence of plasma protein and lipoprotein binding of three pyrethroids for inclusion in physiologically-based pharmacokinetic models of pyrethroids for use in health risk assessments of the insecticides in children and adults.

1. Introduction

The use of pyrethroid insecticides has increased dramatically in the U.S. and European Union with the banning of most organochlorines and the subsequent phase-out of organophosphates (Power & Sudakin, 2007). Permethrin, a mixture of its cis (CIS) and trans (TRANS) isomers, is the most widely-used insecticide in the U.S. (Barr et al., 2010). Pyrethroids are utilized agriculturally to control insects on many crops, landscaping nurseries and gardens. As a result, low levels of the chemicals are present in some fruits, vegetables and grains (Lu, Schenck, Pearson, & Wong, 2010). Pyrethroids are also commonly used for indoor pest control. Certain pyrethroids are even applied to pets and humans to treat head lice, mites, and scabies. Thus, large segments of the populace in North America and Europe are exposed to these compounds, although at quite low levels (Barr et al., 2010; Fortin, Bouchard, Carrier, & Dumas, 2008; Heudorf, Angerer, & Drexler, 2004). Infants and young children are exposed in their homes and daycare centers, primarily by dietary ingestion and hand-to-mouth activity (Becker et al., 2006; Morgan et al., 2007).

Remarkably little is known about the pharmacokinetics (PK) of pyrethroids, other than their metabolism. Absorption, plasma protein binding, distribution, blood–brain barrier penetration, membrane transport and/or elimination could also be important determinants of the target organ (i.e., brain) dose of unmetabolized pyrethroid, and its ensuing neurological effects. PK processes that are found to be important may or may not vary with age. Plasma protein binding of many drugs is known to be lower in neonates and infants, though there is very little information on the nature or extent of pyrethroid binding in any age-group. A significant reduction in plasma protein binding would result in a higher fraction of chemical available for uptake into the brain (Grandison & Boudinot, 2000).

There are a number of techniques utilized to measure plasma protein binding of drugs, but none appear to be suitable for such highly lipophilic compounds as pyrethroids. Equilibrium dialysis, conventional ultrafiltration and ultracentrifugation, the most frequently-employed methods, are unsuitable for two primary reasons: (1) pyrethroids are practically insoluble in the aqueous media that are used; and (2) pyrethroids avidly adhere to glass and polymer components of the necessary separation devices. Many research groups have reported difficulties with adherence or non-specific binding (NSB) of lipid soluble drugs (e.g., corticosteroids, cyclosporine, propranolol) to glass, plastics and

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http://dx.doi.org/10.1016/j.vascn.2014.06.002
1056-8719/

membrane filters. Some investigators have described modifications of techniques to minimize NSB, when assessing plasma binding of relatively lipophilic drugs (Banker, Clark, & Williams, 2003; Henricsson, 1987; Lee et al., 2003; Taylor & Harker, 2006). These approaches appear to be of limited utility, however, with chemicals that are even more hydrophobic.

In light of the foregoing, the objective of the current project was to develop a relatively simple, reliable method for quantifying the extent of binding of three common pyrethroids (DLM, CIS and TRANS) to plasma proteins and total lipoproteins in small-volume samples. An important goal was to verify the method's ability to differentiate between binding to these two major plasma components. Such a method was needed for subsequent investigations of the age- and species-dependency of pyrethroid binding and inclusion of binding parameters in physiologically-based pharmacokinetic (PBPK) models for prediction of brain dosimetry in rodents and humans of different ages.

2. Methods

2.1. Chemicals

Radiolabeled [^{14}C]-deltamethrin (DLM) (54.1 mCi/mmol) was kindly supplied by Bayer CropScience (Stilwell, KS, USA). A 50:50 (CIS:TRANS) radiolabeled permethrin mixture was synthesized by Quotient Radiochemicals, Ltd (Cardiff, UK). It was separated into its CIS (61 mCi/mmol) and TRANS (61 mCi/mmol) isomers by Symbiotic Research (Mount Olive, NJ, USA). The complete chemical names, structures and position of ^{14}C labeling of the radioisotopes are illustrated in Fig. 1. Unlabeled DLM (purity = 98.8%) and, CIS (purity = 99.0%)/TRANS (purity = 99.3%) were kindly provided by Bayer CropScience (Stilwell, KS, USA) and FMC Agricultural Products Group (Princeton, NJ, USA), respectively. ^{3}H-Cyclosporine (0.25 mCi/mmol) and ^{14}C-diazepam (55 mCi/mmol) were supplied by American Radiochemicals (St. Louis, MO, USA). Acetonitrile (HPLC-grade), hexamethyldisilazane (Reagent-grade) and sodium fluoride (NaF) (purity, 99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Isooctane (purity, 99.0%) and 2-octanol (laboratory-grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

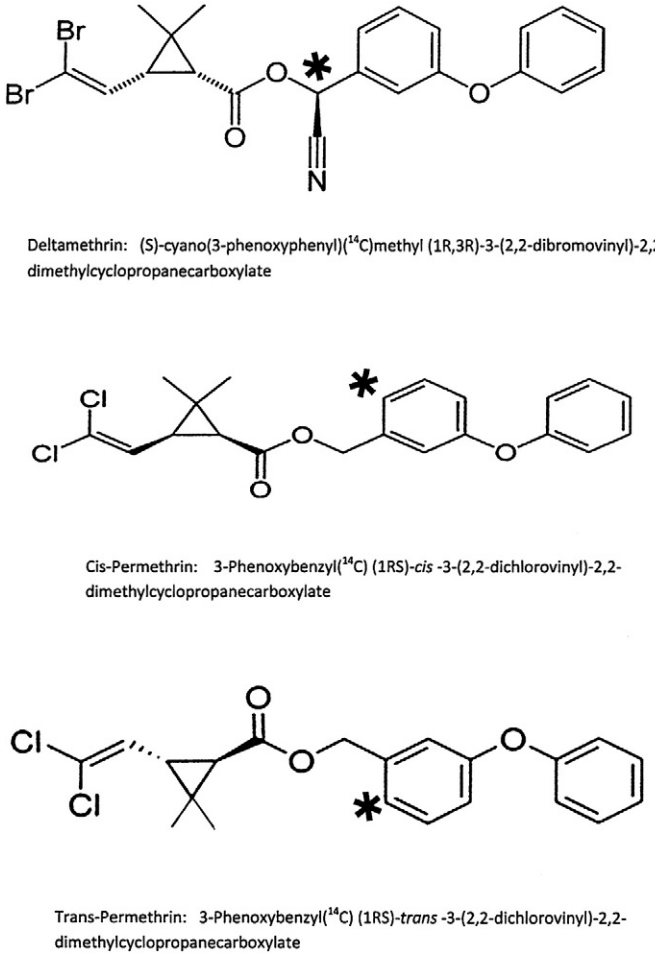

Fig. 1. Chemical names, structures and positions of ^{14}C label of pyrethroids studied in the project.

2.2. Plasma

Frozen, heparinized, pooled adult human and rat plasma were obtained from Innovative Research (Novi, MI, USA). The plasma was stored at −80 °C until use. Thawed plasma was filtered through a 0.45-μm Millipore® filter to remove precipitated fibrinogen.

2.3. Non-specific binding (NSB) experiment

As mentioned in the Introduction, pyrethroids were observed to adhere to, or bind nonspecifically to glass and plastic surfaces. Silanization was accomplished by bathing the tubes in 5% hexamethyldisilazane for 20 min and then drying them overnight in an oven. In order to compare the extent of NSB of DLM, CIS and TRANS to plastic, 250 nM of each ^{14}C-radiolabeled compound in Hank's Balanced Salt Solution (HBSS) buffer was incubated for intervals of up to 120 min in plastic cell culture plates. In each instance, the percent bound to a plate was determined by measuring the total amount of radioactivity remaining in the buffer after each incubation.

2.4. Attainment of plasma protein binding steady-state

An experiment was conducted to learn when binding of pyrethroids reached equilibrium, or steady-state. Ninety-microliter aliquots of human plasma were incubated with 10 μl of 0.64M NaF and ^{14}C-labeled DLM, CIS or TRANS (250 nM final concentration) in a silanized glass vial for periods of up to 180 min. Each timed sample was then mixed with 200 μl of acetonitrile and vortexed for 30 s. The acetonitrile layer was aspirated, mixed with 3 ml of scintillation fluid, and counted in the liquid scintillation counter.

2.5. Technique for quantification of binding to plasma lipoproteins and proteins

Binding of selected pyrethroids was quantified by a 3-step organic solvent extraction procedure (Fig. 2). Stock solutions of ^{14}C-DLM, ^{14}C-CIS and ^{14}C-TRANS were prepared in HBSS at a final concentration of 2.5 μM. Eighty microliters of human and rat plasma were spiked with 10 μl of a range of concentrations (250 nM to 100 μM) of each radiolabeled pyrethroid in silanized glass vials. LoBind® plastic pipette tips (Eppendorf, Hamburg, Germany) were used to avoid pyrethroid adherence. The spiked plasma was immediately treated with 10 μl of 0.64 M NaF to inhibit serum carboxylesterases. The samples were incubated for 3 h in an orbital shaker (110 rpm) at a pH of 7.4 at 37 °C. Following the incubation, 200 μl of isooctane were vortexed for 30 s with each sample and centrifuged for 1 min at 5000 rpm. The isooctane layer was then removed, mixed with 3 ml of scintillation fluid, and counted. The amount of ^{14}C-pyrethroid present in this aliquot was considered to be the unbound portion, or fraction. The remaining plasma was next mixed with 200 μl of 2-octanol, vortexed for 30 s, and centrifuged for 1 min at 5000 rpm. The 2-octanol layer was then removed, mixed with 3 ml of scintillation fluid and counted. The amount of radiolabel in the 2-octanol was considered to be the lipoprotein-bound fraction of pyrethroid. Finally, the remaining plasma was mixed with 200 μl of acetonitrile, vortexed for 30 s, and centrifuged as