Diphenhydramine has Similar Interspecies Net Active Influx at the Blood–Brain Barrier

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ABSTRACT: In rats, oxycodone, diphenhydramine, and [4-chloro-5-fluoro-2-(3-methoxy-2-methyl-phenoxy)-benzyl]-methylamine (CE-157119) undergo net active influx at the blood–brain barrier (BBB) based on significantly greater interstitial fluid compound concentrations (C_{ISF}) than unbound plasma compound concentrations ($C_{p,u}$). Oxycodone and diphenhydramine have C_{ISF}:$C_{p,u}$ of 3.0 and 5.5, respectively, while CE-157119 has an unbound brain compound concentration ($C_{b,u}$):$C_{p,u}$ of 3.90; $C_{b,u}$ is a high-confidence C_{ISF} surrogate. However, only CE-157119 has published dog and nonhuman primate (nhp) neuropharmacokinetics, which show similar $C_{b,u}$:$C_{p,u}$ (4.61 and 2.04, respectively) as rats. Thus, diphenhydramine underwent identical interspecies neuropharmacokinetics studies to determine if its net active BBB influx in rats replicated in dogs and/or nhp. The single-dose-derived rat $C_{b,u}$:$C_{p,u}$ (3.90) was consistent with prior steady-state-derived C_{ISF}:$C_{p,u}$ and similar to those in dogs (4.88) and nhp (4.51–5.00). All large animal interneurocompartmental ratios were ≤1.8-fold different than their rat values, implying that diphenhydramine has constant and substantial $C_{b,u}$-favoring disequilibria in these mammals. Accordingly, the applied $C_{b,u}$-forecasting methodology accurately predicted [estimated mean (95% confidence interval) of 0.84 (0.68, 1.05)] $C_{b,u}$ from each measured $C_{p,u}$ in large animals. The collective datasets suggest these $C_{b,u}$-preferring asymmetries are mediated by a species-independent BBB active uptake system whose identification, full characterization, and structure–activity relationships should be prioritized for potential exploitation. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1557–1562, 2014

Keywords: blood–brain barrier; CNS; diphenhydramine; drug disposition; drug transport; preclinical pharmacokinetics

INTRODUCTION

Growing evidence[1,2] continues to support unbound brain compound concentration ($C_{b,u}$) as a high-confidence and efficiently derived surrogate of interstitial fluid (ISF) compound concentration (C_{ISF}), which best defines neurotherapeutic exposure–response relationships.[3,4] Hence, the ability to predict reliably large animal C_{ISF}, which is rarely directly confirmed because of the impracticability of ISF collection in dogs and primates, would help ensure experimental molecules adequately test neuropharmacological hypotheses in higher order species, most notably humans. A recent report[5] on small-molecule neuropharmacokinetics evaluated using acute dose, rat-derived $C_{b,u}$-to-unbound plasma compound concentration ratios ($C_{b,u}$:$C_{p,u}$) to forecast correctly dog and nonhuman primate (nhp) $C_{b,u}$ from their measured total plasma compound concentration (C_p) and species-specific unbound plasma fraction ($f_{u,p}$). In that study, [4-chloro-5-fluoro-2-(3-methoxy-2-methyl-phenoxy)-benzyl]-methylamine (CE-157119) had in rats a 2.65- and 3.90-fold asymmetry favoring $C_{b,u}$ over its cerebrospinal fluid (CSF) compound concentration (C_{CSF}) and $C_{p,u}$, respectively; similar $C_{b,u}$:C_{CSF} (>2.72) and $C_{b,u}$:$C_{p,u}$ (≥2.04) disequilibria existed in both dogs and nhp. For CE-157119, the translatability of its rat-derived $C_{b,u}$:$C_{p,u}$ allowed the accurate prediction of its $C_{b,u}$ from measured C_p in dogs (≤1.9-fold different from observed $C_{b,u}$) and nhp (<2.9-fold divergent). Significantly, these data revealed that CE-157119 experiences similar net active influx at the blood–brain barrier (BBB) in rats, dogs, and nhp.

Small-molecule drugs recognized to undergo net active uptake across the BBB are very uncommon. To our knowledge, only oxycodone and diphenhydramine are reported to have rat-derived C_{ISF}:$C_{p,u}$ disequilibria of 3.0[6] and 5.5,[7] respectively, and this has been attributed to a yet-identified transporter that governs pyrilamine influx at the BBB in rats.[7–9] Based on the species-independent $C_{b,u}$-favoring neuropharmacokinetics of CE-157119[5] and the presence of the putative pyrilamine transporter in rat,[8] bovine,[10] and human[11] brain capillary endothelial cells (BCEC), it is important to determine for oxycodone and diphenhydramine if their net active BBB influx in rats is replicated in dogs and/or nhp. If so, this would strongly suggest this particular uptake carrier is retained in the mammalian hierarchy, which would have important BBB-based drug design implications. Already, diphenhydramine has been found to have a C_{ISF}:$C_{p,u}$ of 2–3 in sheep.[12] Such work is further legitimized by studies[5,13] with P-glycoprotein substrates that show brain penetration differences between rats and large animals implying possible inconsistent BBB properties across species. Because

Abbreviations used: $C_{b,u}$, unbound brain compound concentration; C_{ISF}, interstitial fluid compound concentration; $C_{p,u}$, unbound plasma compound concentration; C_{CSF}, cerebrospinal fluid compound concentration; AUC, area under the matrix compound concentration–time curve; $C_{b,u,obs}$, large animal observed unbound brain compound concentration; $C_{b,u,pred}$, large animal predicted unbound brain compound concentration.

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oxycodone is a controlled substance and diphenhydramine has well-characterized rat neuropharmacokinetics,[7,14,15] diphenhydramine was selected for neuropharmacokinetics studies whose primary purpose was to determine if it has a consistent $C_{b,u}$:$C_{p,u}$ in rats, dogs, and nhp. A regular $C_{b,u}$-preferring asymmetry would further suggest both a retained species-independent active influx system (presumably that of pyrilamine) at the mammalian BBB and that diphenhydramine and CE-157119 (along with oxycodone and other select lipophilic basic amines)[10,16] are probably common substrates for it, which could ultimately be exploited to enhance clinical neurotherapeutic brain penetration. A secondary experimental goal was to compare all neuropharmacokinetic parameters of diphenhydramine across the three species. Together, these objectives further evaluated for small-molecule medicines the ability to project accurately their large animal $C_{b,u}$ from an experimentally determined C_p, species-specific $f_{u,p}$, and rat-derived $C_{b,u}$:$C_{p,u}$.[3]

EXPERIMENTAL

Chemicals and Reagents

Diphenhydramine [2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine] hydrochloride was synthesized (>99% chemical purity) and characterized at Pfizer Worldwide Research and Development (WRD, Groton, Connecticut). Species-specific control plasma was procured from Bioreclamation Inc. (Hicksville, New York), and artificial CSF (an aqueous solution of 147 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, and 1.3 mM $CaCl_2$) and Sprague–Dawley rat brain tissue were obtained at WRD. Chemicals and solvents of reagent or HPLC grade were supplied by Aldrich Fine Chemical Company (Milwaukee, Wisconsin), Fisher Scientific (Pittsburgh, Pennsylvania), Thermo Fisher Scientific (Waltham, Massachusetts), and Mallinckrodt Baker (Phillipsburg, New Jersey). All animal studies were performed in compliance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) utilizing protocols reviewed and approved by the WRD Institutional Animal Care and Use Committee. The in-life and bioanalytical portions of the rat or large animal studies were performed at BioDuro, Pharmaceutical Product Development Inc. (Beijing, China) or WRD, respectively. Harvested biomatrices were stored at −20°C or −70 °C until processing.

Plasma and Brain Homogenate Nonspecific Binding

Species-specific $f_{u,p}$ and Sprague–Dawley rat brain homogenate unbound fraction ($f_{u,b}$) were determined by equilibrium dialysis (1 μM diphenhydramine, N = 6/species) as previously described.[5]

Neuropharmacokinetics Studies

Rat

Each male Sprague–Dawley rat (250–300 g, N = 3/time point) received a single subcutaneous (SC) injection (5 mL/kg) of diphenhydramine (20 mg/kg)[17] in phosphate-buffered saline (PBS, pH 7.4) (4 mg/mL). Blood, CSF, and whole brain were collected as detailed[5] from each rat placed under isoflurane anesthesia at 0.25, 0.5, 1, 2, 4, 7, or 24 h after the dose.

Large Animals

Non-naïve female beagle dogs and male Cynomolgus monkeys (nhp), considered being in good health as judged by overall condition (asymptomatic) and medical history by Veterinary Medicine (WRD), were obtained from WRD animal colonies by written permission for participation in neuropharmacokinetics studies. Large animal euthanasia, sample collection, and sample processing precisely followed published procedures.[5] Dogs (N = 8) received diphenhydramine (2 mg/kg, SC) in PBS (2 mg/mL, 1 mL/kg), and whole blood, CSF, and brain region samples (frontal cortex, occipital cortex, thalamus, caudate, and putamen) were collected at 0.5, 1, 2, or 4 h after the dose (N = 2/time point). Nonhuman primates received either 3.2 (N = 3) or 10 mg/kg, SC (N = 4) of diphenhydramine in PBS (3.2 or 10 mg/mL, 1 mL/kg) with biomatrices (brain regions were frontal cortex, temporal cortex, occipital cortex, cerebellum, thalamus, hypothalamus, caudate, putamen, hippocampus, and substantia nigra) collected at either 1, 2, or 6 h after the dose or 0.5, 1, 2, or 6 h after the dose, respectively.

Quantification of Diphenhydramine in Biological Matrices

The quantification of diphenhydramine within biomatrices collected from rats occurred at BioDuro, Pharmaceutical Product Development Inc. For samples from nonspecific binding experiments and large animals, diphenhydramine quantification was conducted at WRD. For bioanalytical sample preparation, plasma and CSF were used as is, whereas brain tissue were first homogenized in a fourfold volume (w/v) of PBS (BioDuro) or 40% H_2O in *i*PrOH (WRD). All matrices were processed for diphenhydramine quantification using either liquid–liquid extraction (BioDuro) or acetonitrile (CH_3CN)-mediated matrix precipitation (WRD) methodology followed by a characterized liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay. For liquid–liquid extraction, an aliquot (10 μL) of an internal standard dissolved in 1:1 (v/v) CH_3CN:H_2O was added to aliquots of plasma (50 μL), CSF (50 μL), or brain homogenate (100 μL), which were then extracted with methyl *t*-butyl ether (500 μL). Samples were vortex-mixed and centrifuged (3500*g* for 10 min at 4°C) to sediment precipitated matrix constituents, and the respective supernatants were transferred to clean tubes, concentrated under N_2 at 40°C, and the resulting residues were reconstituted in 1:1 (v/v) CH_3CN:H_2O with 0.1% HCO_2H (100 μL) for bioanalytical analysis. For CH_3CN-mediated matrix precipitation, a sample aliquot (50 μL) was diluted with CH_3CN (300 μL) containing an internal standard. Samples were vortex-mixed and centrifuged (3000*g* for 10 min at 4°C) to afford supernatant (250 μL), which was transferred to a 96-well plate, evaporated under N_2 at 37°C and the resulting residues were reconstituted in 1:3 (v/v) CH_3CN:H_2O (100 μL) to provide the bioanalytical sample. Individual standard curves were prepared in respective control matrices, an appropriate dynamic range was achieved for all analytical assays, and instrument settings and potentials were adjusted to optimize the MS signal for diphenhydramine. Samples were analyzed by an LC–MS/MS composed of an AB Sciex API 4000 tandem quadrupole mass spectrometer with a TurboIon Spray probe (AB Sciex Inc., Ontario, Canada), tertiary Shimadzu LC20AD pumps (Shimadzu Scientific Instruments, Columbia, Maryland), and a CTC PAL autosampler (Leap Technologies, Carrboro, North Carolina). All raw data were processed using Analyst Software version 1.4.2 or 1.5.2 (AB Sciex Inc.). The