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Unbound Drug Concentration in Brain Homogenate and Cerebral Spinal Fluid at Steady State as a Surrogate for Unbound Concentration in Brain Interstitial Fluid

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Abbreviations used are: BBB, blood-brain barrier; BCSFB, blood cerebrospinal fluid barrier; C_m, unbound brain interstitial fluid concentration measured by brain microdialysis; C_{ub}, unbound brain concentration measured by brain homogenate method; C_{up}, unbound plasma concentration; C_{CSF}, CSF concentration; P-gp, P-glycoprotein; KO, knockout; WT, wild type.

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Abstract

The objective of the present study was to examine the accuracy of using unbound brain concentration determined by a brain homogenate method (C_{ub}), cerebral spinal fluid concentration (C_{CSF}), and unbound plasma concentration (C_{up}) as a surrogate for brain interstitial fluid concentration determined by brain microdialysis (C_m). Nine compounds: carbamazepine, citalopram, ganciclovir, metoclopramide, N-desmethylclozapine, quinidine, risperidone, 9-hydroxyrisperidone, and thiopental were selected and each was administered as an intravenous bolus (up to 5 mg/kg) followed by a constant intravenous infusion (1-9 mg/kg/hr) for 6 hours in rats. For 8 of the 9 compounds, the C_{ub} were within 3-fold of their C_m; thiopental had a C_m of 4-fold of its C_{ub}. The C_{CSF} of 8 out of the 9 compounds were within 3-fold of their corresponding C_m; 9-hydroxyrisperidone showed a C_{CSF} of 5-fold of its C_m. The C_{up} of 5 out of the 9 compounds were within 3-fold of their C_m; 4 compounds (ganciclovir, metoclopramide, quinidine, and 9-hydroxyrisperidone) had C_{up} of 6- to 14-fold of their C_m. In conclusion, the C_{ub} and C_{CSF} were within 3-fold of the C_m for the majority of the compounds tested. The C_{up} were within 3-fold of C_m for lipophilic non-P-glycoprotein substrates and greater than 3-fold of C_m for hydrophilic or P-glycoprotein substrates. The present study indicates that the brain homogenate and CSF methods may be used as surrogate methods to predict brain interstitial fluid concentrations within 3-fold of error in drug discovery and development settings.

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Introduction

For drugs with an intended action in the central nervous system it is assumed that unbound drug in brain interstitial fluid is in direct contact or in equilibrium with the site of action (de Lange and Danhof, 2002). Therefore, in preclinical and clinical pharmacokinetic/pharmacodynamic studies, it is critical to determine the concentration in the interstitial fluid for brain-targeted compounds. Unbound plasma concentration (C_{up}) has been used to represent the unbound concentration in tissue (Wilkinson, 2001). Because the brain is separated from the plasma by the blood-brain barrier (BBB) and the blood cerebrospinal fluid barrier (BCSFB), C_{up} may not represent the interstitial fluid concentration (Davson and Segal, 1995; Hammarlund-Udenaes et al., 2008; Liu et al., 2008).

Microdialysis has been considered as a standard approach to measure interstitial fluid concentration (C_m) (Joukhadar and Muller, 2005; Chaurasia et al., 2007). Although this technique has been developed for more than two decades, it is primarily used for determination of neurotransmitters and not drug concentrations in the brain. The main limitations of this technique include high resource requirements, low throughput, and special surgical skills to set up the experiment. In addition, many compounds in the discovery stage are often very lipophilic and it is difficult to apply microdialysis technique to study these compounds due to high non-specific binding. Importantly due to ethical reasons, this method cannot be used routinely to measure the interstitial fluid concentration in clinical trials although it has been used to monitor glucose metabolism such as lactate and pyruvate ratio as a marker for ischemia in brain trauma patients in a few life threatening situations (Benjamin et al., 2004; Hillered et al., 2006; Chaurasia et al., 2007; Helmy et al., 2007). Because of these limitations, several alternative methods, such as brain homogenate, brain slice and CSF, have been proposed and used to estimate brain unbound drug concentrations (Liu et al., 2008).

A brain homogenate method has been proposed as a surrogate approach to estimate brain unbound concentration (C_{ub}) (Kalvass and Maurer, 2002). In this approach the unbound fraction in brain tissue is estimated by the unbound fraction in brain homogenate, which is determined using equilibrium dialysis or ultracentrifugation. Several indirect validation studies

have been published, such as comparing the projected brain to plasma concentration ratios with the observed ratios, or comparing the unbound brain fraction determined by brain homogenate method with that determined by brain slice method, or by CSF method (Kalvass and Maurer, 2002; Maurer et al., 2005; Becker and Liu, 2006; Liu et al., 2006; Summerfield et al., 2006; Summerfield et al., 2008). Recently, Friden at al. (2007) demonstrated that C_{ub} predicted C_m within 3-fold of error for 10 out of 15 compounds. In that study, the microdialysis for 14 of the 15 compounds were compiled from the literature and all the data were generated using different methods or in different species.

Another commonly used method in both preclinical and clinical studies is the CSF concentration (C_{CSF}). Although CSF is in direct contact with brain tissue and there are discontinuous gap junctions found in most areas of the ventricular ependyma, C_{CSF} may not always represent the interstitial fluid concentration because of the convective bulk flow of brain interstitial fluid from brain tissue into CSF, and different transporters expressed at the BBB and BCSFB (de Lange and Danhof, 2002; Shen et al., 2004). However, a pragmatic question is how large is the difference between CSF concentration and the C_m.

Studies have not been reported to examine and compare the C_{ub}, C_{CSF}, C_{up}, and C_m for multiple compounds under identical experimental conditions. The objective of the present study was to compare the accuracy of using C_{ub}, C_{CSF}, and C_{up} as a surrogate to predict C_m. We selected 9 model compounds representing different physicochemical properties and various P-glycoprotein (P-gp) transport activities. The results from this study will help us to understand whether the C_{ub}, C_{CSF}, and C_{up} can be used as surrogates to predict the interstitial fluid concentration in drug discovery and development setting and identify potential caveats of these methods.

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Materials and Methods

Chemicals. Carbamazepine, metoclopramide, N-desmethylclozapine, quinidine, and thiopental were obtained from Sigma-Aldrich (St. Louis, MO). Risperidone and 9-hydroxyrisperidone were obtained from SynFine Research (Richmond Hill, Ontario, Canada). Citalopram and ganciclovir were synthesized at Roche Palo Alto, LLC (Palo Alto, CA) with purity greater than 98%. All other chemicals used in the experiments were of the highest available grade.

Brain Microdialysis. Jugular and femoral-cannulated male Sprague Dawley rats (250-350 g), with surgically implanted a microdialysis guide cannula and a dummy probe (CMA/12, CMA Microdialysis, Solna, Sweden), were purchased from Charles River Laboratories (Hollister, CA). The guide cannula was implanted in the prefrontal cortex of rat brain using a stereotaxic instrument at 3.2 mm anteroposterior, 1.0 mm mediolateral, and 0.5 mm dorsoventral to the bregma point and secured to the skull with screws and dental cement. The animals were acclimatized to the laboratory environment for 3-5 days before the study. At approximately 16 hrs prior to dosing, the rats were placed into individual BASi RATURN systems (Bioanalytical Systems, Inc., W. Lafayette, IN) for freely moving animals with food and water *ad libitum*. The dummy probes were replaced with CMA 12/2 mm probes (CMA Microdialysis, Solna, Sweden), and perfused with an artificial CSF solution (147 mM NaCl, 2.7 mM KCl, and 1.2 mM $CaCl_2$) at 1 µL/min overnight using microdialysis pumps (CMA/102, CMA Microdialysis, Solna, Sweden). On the day of the study, the outlets were connected to the BASi Refrigerated Honeycom Fraction Collector (Bioanalytical Systems, W. Lafayette, IN) at 4°C and perfused at 1 µL/min. Rats (n=5-6) received an intravenous bolus dose followed by intravenous infusion via the femoral cannula for carbamazepine (1.5 mg/kg and 1 mg/kg/hr), citalopram (10 mg/kg and 3 mg/kg/hr), ganciclovir (0 mg/kg and 3 mg/kg/hr), metoclopramide (0.5 mg/kg and 1 mg/kg/hr), N-desmethylclozapine (1 mg/kg and 1.33 mg/kg/hr), quinidine (5 mg/kg and 9 mg/kg/hr), risperidone (0.5 mg/kg and 1 mg/kg/hr), and thiopental (0.35 mg/kg and 1 mg/kg/hr). Compound 9-hydroxyrisperidone was monitored after dosing its parent drug risperidone (0.5 mg/kg and 1 mg/kg/hr). Carbamazepine was prepared in 2-hydroxypropyl-ß-cyclodextrin; and N-desmethylclozapine was prepared in

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0.5% dextrose at pH 8. All other compounds were prepared in saline. Blood samples were collected via jugular vein cannula at 0.25, 1, 2, 4, 5, and 6 hour post start of infusion for ganciclovir, at 2, 4, 5, and 6 hour for citalopram, and at 1, 2, 4, 5, and 6 hour for the remaining compounds. Blood was then centrifuged to obtain plasma. The perfusate samples were serially collected from each animal at 0.5 hour intervals from 1 hour pre-dose to 6 hours post-dose. At 6 hour, animals were euthanized by carbon dioxide asphyxiation. Approximately 100 µL CSF samples were collected via cisterna magna puncture from each rat. Brain samples were also collected. All the samples were stored at –20°C prior to analysis.

In Vitro Recovery of the Microdialysis Probe. The in vivo recovery of the microdialysis probe for each compound was estimated using in vitro microdialysis by a gain method. A CMA 12/2 mm probe was immersed in the artificial CSF solution containing 100 ng/mL of testing compound in a 1.5-mL tube at 37°C and perfused with the artificial CSF solution at 1 µl/min for 4 hours. The dialysate was collected every 0.5 hour interval and stored at –20°C prior to analysis. The ratio of the concentration in the dialysate versus that in the solution was calculated as the in vitro recovery.

Protein Binding. The in vitro unbound fraction in brain homogenate and plasma for each compound was determined using a 48-well Rapid Equilibrium Dialysis (RED) device (Linden Bioscience, Woburn, MA). Brain tissue was homogenized in two volumes (w/v) of 0.9% saline. Brain homogenate or plasma was spiked with a compound for a concentration of 1000 ng/mL. Two-hundred µL of the matrix was added to the donor side of a dialysis chamber. The receiver side contained 350 µL of the Sorenson's buffer. The dialysis apparatus was maintained on a shaking device at 37°C for 4 hours. The drug concentrations were determined as described below.

Sample Analysis. The brain tissues were homogenized in two volumes (w/v) of 0.9% saline. Fifty µL of brain homogenate or plasma and 200 µL of internal standard in acetonitrile were mixed in 96-well polypropylene plates. For the protein binding samples, 25 µL of diluted brain homogenate or plasma samples were mixed with 25 µL of control buffer; and 25 µL of buffer samples mixed with 25 µL control brain homogenate or plasma to yield identical matrix between

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donor and receiver side of samples. The samples were then mixed with 150 µL of acetonitrile containing internal standard. The acetonitrile mixtures were vortexed and then centrifuged at 1,800g for 10-15 minutes. Aliquots of the supernatant were transferred to a 96-well plate, and diluted with equal volume of water prior to analysis by high performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS). Aliquots of the CSF (30 µL) or perfusate samples were mixed with 20 µL of internal standard solution in acetonitrile in 96-well glass tubes and analyzed by HPLC-MS/MS.

The standard curves were prepared by spiking a known amount of compound into blank matrix and then processing according to procedure described previously for each matrix. The HPLC-MS/MS system consisted of either a Shimadzu ternary pump (Shimadzu LC-10A, Kyoto, Japan) or an Agilent quaternary pump HPLC system (Hewlett Packard, Palo Alto, CA), an HTS-PAL autosampler (Leap Technologies, Switzerland) and a PE Sciex API 4000 (Perkin-Elmer Sciex Instruments, Foster City, CA) mass spectrometer with a turbo ion spray interface (PE-Sciex, Thornhill, Ontario, Canada). A 10-µL aliquot of each sample was injected onto a reverse-phase column. The HPLC-MS/MS conditions for the 9 compounds can be found in Table 1. The concentration of all samples was within the linear range of quantitation for all assays. The low limit of quantitation for the 9 compounds was 0.5-2 ng/mL for plasma, 1-7.5 ng/g for brain, and 0.05-0.25 ng/mL for CSF and the dialysate. The assay accuracy was between 80% and 120%.

Data Analysis. The unbound fractions determined from diluted brain tissue homogenates were corrected to yield an estimate of unbound fraction in the intact brain tissue using Equation 1 (Kalvass and Maurer, 2002).

$$f_{u,brain} = \frac{1}{1 + (1/f_{u,homogenate} - 1) \bullet Dilution} \qquad \text{Equation 1}$$

Where $f_{u,brain}$ and $f_{u,homogenate}$ represent the unbound fraction in brain tissue and unbound fraction in brain homogenate. Dilution is the dilution factor for the brain homogenate.

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Results

The compounds selected in the present study include acidic and basic, and neutral compounds with diverse structures and physicochemical properties (Table 2). The recovery of the microdialysis probes was determined in vitro by measuring the gain in the dialysis solution for each compound and ranged from 18.3 ± 4.1% to 55.2 ± 5.7% (mean ± SD) with an average of the recovery of 34.6 ± 11.0% (Table 3). The unbound fractions of each compound in rat plasma and brain were determined using equilibrium dialysis. The rat plasma unbound fractions ranged from 0.0625 ± 0.0047 to 0.9720 ± 0.319. The rat brain tissue unbound fraction ranged from 0.00569 ± 0.00030 to 0.855 ± 0.546 (Table 3). The brain unbound fractions were equal to or lower than the unbound fraction of plasma for this set of compounds.

To study the relationship of drug concentrations in different compartments, the studies were designed to reach steady state for both the plasma and brain concentrations. As demonstrated in Figure 1, the concentration in plasma and brain compartments reached steady state at 1 to 4 hours post the start of infusion. All of the analyses of the plasma, brain, CSF and interstitial fluid concentration were based on the concentrations at 6 hours after the start of infusion (Table 3 and Figure 2).

The fold of difference of the C_{ub}, C_{CSF}, and C_{up} over C_m at 6 hours after the start of infusion are shown in Table 4 and the relationship between C_{ub}, C_{CSF}, or C_{up} and C_m are presented in Figure 2. For 8 of the 9 compounds, their C_{ub} were within 3-fold of their C_m. The C_m of thiopental was 4-fold of its C_{ub} (Figure 2A and Table 4). For 8 of the 9 compounds, their C_{CSF} were within 3-fold of their C_m. The C_{CSF} of 9-hydroxyrisperidone was 5-fold of its C_m (Figure 2B and Table 4). The C_{up} of the 9 compounds was equal to or greater than their C_m (Figure 2C and Table 4). For 5 of the 9 compounds, their C_{up} were within 3-fold of their C_m. The C_{up} of ganciclovir was 14-fold of its C_m.

In the present study, compounds whose ratio of brain/plasma concentration in mdr1a/1b knockout mice over the brain/plasma concentration in wild type mice (KO/WT) greater than 2 are defined as P-gp substrates. Among the 9 compounds, 5 compounds are P-gp substrates and the rest are non-P-gp substrates or their P-gp transport activities are not known (Table 2). For those

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P-gp substrates, the range of the KO/WT ratios is 6.6-36. For non-P-gp substrates, the range of KO/WT ratios is 1.1-1.9. The KO/WT ratios correlated C_{up}/C_m ratios for the 7 compounds (r = 0.89, Figure 3A). The KO/WT was within 3-fold of C_{up}/C_m for 5 of the 7 compounds. The KO/WT ratios for the other 2 compounds, quinidine and risperidone, were 5- and 6-fold greater than their C_{up}/C_m ratios, respectively. The KO/WT ratios also correlated C_{up}/C_{ub} ratios for the 7 compounds (r = 0.78, Figure 3B). The KO/WT was within 3-fold of C_{up}/C_{ub} for 6 of the 7 compounds. The C_{up}/C_{ub} for thiopental was 4-fold of its KO/WT.

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Discussion

The major findings in the present study are (1) the C_{ub} predicted the C_m within 3-fold of error for 8 of the 9 compounds and 4-fold for 1 of the 9 compounds; (2) the C_{CSF} predicted the C_m within 3-fold of error for 8 of the 9 compounds and 5-fold for 1 of the 9 compounds; (3) the C_{up} predicted the C_m within 3-fold of error for 5 of the 9 compounds and over predicted the C_m for the other 4 compounds (6- to 14-fold). These results support the use of brain homogenate or CSF as a surrogate for the interstitial fluid concentration in drug discovery and development settings.

The brain homogenate predicted steady state brain unbound concentration for 8 out of 9 compounds within 3-fold of error for the C_m. Only thiopental showed 4-fold difference. This correlation is better than the results reported recently by Friden et al (2007) who showed that C_{ub} predicted C_m within 3-fold for 10 of the 15 compounds and the other 5 compounds showed approximately a 5-fold of error. In that study, the microdialysis data for all the compounds except for CP-122721 were collected from the literature and generated under very different experimental conditions, including recovery methods (in vitro and in vivo), microdialysis probe locations, animal species (rats or rabbits), and dosing routes. For CP-122721, the in vivo microdialysis and in vitro brain homogenate studies were carried out in the same laboratory and its C_{ub} and C_m was very similar.

Because the brain homogenate method can be much more easily implemented in the drug discovery setting, the present study only focused on the evaluation of the brain homogenate method. The concern for the brain homogenate method is that brain homogenization may change the drug binding properties by destroying cell structure and unmasking binding sites that are not accessible to a drug in vivo. In addition, the unbound fraction in brain tissue may not be extrapolated from the unbound fraction in the diluted brain tissue homogenate if the drug only presents in the interstitial space. In our previous work, we demonstrated that the brain homogenate method was similar to brain-slice method in prediction of C_m (Becker and Liu, 2006). This conclusion is also supported by the data reported by Friden at el. (2007). The unbound fraction, a reciprocal of the unbound volume being reported in the paper, determined by the brain

homogenate method and by the brain slices method were within 3-fold for 15 compounds except for one compound, gabapentin. This compound showed 4-fold difference (Friden et al., 2007).

CSF concentration has been considered closely related to the interstitial fluid concentration and used as a surrogate for the interstitial fluid concentration in preclinical and clinical studies. CSF is separated from blood by BCSFB and is in direct contact with brain tissue. The ependymal lining of the ventricles allows diffusional and convectional exchange with the brain interstitium (Abbott, 2005). Because the drug transporters at the BBB and BCSFB are different, the C_{CSF} is not necessarily identical to the interstitial fluid concentration but it is not clear much difference exists between C_{CSF} and the interstitial fluid concentration and the potential errors of using C_{CSF} as surrogate for the interstitial fluid concentration. In the present study, we observed that C_{CSF} was within 3-fold of error for 8 of the 9 compounds and 5-fold for the other compound. These results support that C_{CSF} may be used as a surrogate in drug discovery and development to predict the interstitial fluid concentration in the brain.

CSF as a surrogate for the interstitial fluid concentration is supported by the data presented by Shen et al. (2004) who compiled C_{CSF} and C_m for 20 compounds. The C_{CSF} was within 3-fold of error for 17 of the 20 compounds and 4- to 5-fold for 2 of the 17 compounds (Liu et al., 2006). Only morphine-6-glucuronide showed large discrepancy between C_{CSF} and C_m. The C_{CSF} of morphine-6-glucuronide was 19 fold lower than its C_m. This discrepancy may be due to the experimental conditions where C_{CSF} and the interstitial fluid concentration did not reach equilibrium as the brain half-life is longer its plasma half-life (Bouw et al., 2001). Experimental variability might also contributed to the discrepancy because the C_{CSF} and C_m data were obtained from separate studies (Stain-Texier et al., 1999). The ratio of total brain concentration of morphine-6-glucuronide versus the C_m was 0.11 as reported by Stain-Texier et al. (1999) but was 0.20 as reported by Bouw et al. (2001). Further studies are needed to assess the difference between C_{CSF} and C_m for morphine-6-glucuronide.

Plasma represents the easiest accessible matrix as compared to brain tissue and CSF, therefore, the unbound plasma concentration has been used as the primary surrogate for the interstitial fluid concentration, particularly in clinical studies. Because of the existence of the BBB,

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C_{up} is often equal to or higher than the interstitial fluid concentration (Liu et al., 2008). The results from the present study are consistent with this view. For the 3 lipophilic non-P-gp substrates, their C_{up} was same as their C_m but for the 4 P-gp substrates and the 2 hydrophilic unknown transporter substrates, their C_{up} values were 2-14 fold of their C_m. The results from the present study also confirm our hypothesis that C_{CSF} more accurately predicts the interstitial fluid concentration of the brain than C_{up} does (Liu et al., 2006).

Under the assumption that the only difference between the mdr1a/1b gene knockout and the wild type mice is P-gp at the BBB, the unbound plasma and brain concentration ratio in the wild type mice can be estimated from the KO/WT ratio (Liu et al., 2008). The results from the present study are consistent with this projection. The efflux ratios observed in mdr1a/1b mice correlated the ratios of C_{up}/C_m and C_{up}/C_{ub} in the rat. The KO/WT ratios were within 3-fold of the C_{up}/C_m ratios for 5 of the 7 compounds and within 3-fold of the C_{up}/C_{ub} for 6 of the 7 compounds. Therefore one may use KO/WT ratio to semi-quantitatively estimate the unbound plasma and brain concentration ratio assuming no species difference in P-gp activity at the BBB (Liu et al., 2008).

Risperidone is a P-gp substrate with a KO/WT ratio of 10 in P-gp knockout mice (Doran et al., 2005) but interestingly its C_{up} was only 2- and 4-fold greater than its C_m and C_{ub} in rats, respectively. This finding are consistent with the literature. Summerfield et al. (2006) observed the predicted brain/plasma ratio in rats was 0.76 based on the unbound fraction in plasma and brain tissue. This projected value is within 3-fold of the observed in vivo brain/plasma ratio 0.3, suggesting low or no significant efflux activity at the BBB.

One of the limitations of the present study is that in vitro recovery was used to calibrate the microdialysis probes. In vitro recovery methods may be less accurate compared to the in vivo recovery methods, such as no-net-flux and retrodialysis methods (Lonnroth et al., 1987; Olson and Justice, 1993; Wang et al., 1993). However, there is a trade-off between theoretical requirements for the recovery and practical possibilities when performing microdialysis (Chaurasia et al., 2007). The in vivo recovery methods are resource intensive and thus were not suitable in the drug discovery setting. Since the main goal of this work was to assess which

concentration is a more appropriate surrogate of C_m, the use of in vitro recovery should not impact on our conclusions.

In summary, the present study supports that the C_{ub} and C_{CSF} can be used as a surrogate for the interstitial fluid concentration in drug discovery and development setting. The errors of these methods for most compounds will likely be less than 3-folds. The C_{up} may predict the interstitial fluid concentration for lipophilic and non-efflux substrates but over predict the interstitial fluid concentration for polar or efflux substrates. The future research needs to further assess the utilities and limitations of brain homogenate and CSF methods, and to refine the current surrogate methods to improve their accuracy.

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FIGURE LEGENDS

Figure 1. Rat unbound plasma concentration (solid circles) and brain unbound interstitial drug concentration measured by brain microdialysis (open circles) versus time profiles (Mean ± SD, n = 3-6) of 9 compounds after an intravenous bolus dose and followed by an constant intravenous infusion for 6 hours in rats.

Figure 2. The relationship of brain unbound interstitial drug concentration measured by brain microdialysis (C_m) and brain unbound drug concentration measured by brain homogenate method (C_{ub}, A), CSF concentration (C_{CSF}, B), and unbound plasma concentration (C_{up}, C) at steady state in rats (Mean ± SD, n = 3-6). The C_{ub} were calculated from the total brain concentration and brain unbound fraction. All of the concentrations represent the values at 6 hour post start of an intravenous bolus and followed with an constant intravenous infusion. The solid and dashed lines represent unity and 3-fold boundaries, respectively. Symbols for compounds are defined in Table 1.

Figure 3. The relationship of the ratio of C_{up}/C_m and the ratio of KO/WT (A) and the ratio of C_{up}/C_{ub} and the ratio of KO/WT (B). The solid and dashed lines represent unity and 3-fold boundaries, respectively. Symbols for compounds are defined in Table 1.

Table 1. HPLC-MS/MS conditions for the 9 compounds

Compound	Retention Time (min)	MRM Transition	Initial Gradient Conditions	Final Gradient Conditions	HPLC Column[1]
Carbamazepine (C)	2.3	237.09/194.20	A:B (90:10)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
Citalopram (Ci)	3.0	325.26/109.40	A:B (95:5)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
Ganciclovir (G)	2.2	256.18/152.10	A:B (95:5)	A:B (10:90)	Aquasil C18 50x4.6 mm, 5 µm
Metoclopramide (M)	2.0	301.21/228.10	A:B (90:10)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
N-desmethylclozapine (N)	3.2	313.23/192.00	A:B (95:5)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
Quinidine (Q)	3.0	325.00/160.00	A:B (95:5)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
Risperidone (R)	2.5	411.20/191.30	A:B (90:10)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
9-OH-Risperidone (R9)	2.4	427.25/206.80	A:B (90:10)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm
Thiopental (T)	3.8	241.04/101.00	A:B (90:10)	A:B (10:90)	BDS Hypersil C18 50x2.1 mm, 5µm

MRM, multiple reaction monitoring

A: 0.1% formic acid

B: acetonitrile:methanol:formic acid (49.95:49.95:0.1)

C: acetonitrile: formic acid (99.9:0.1)

[1]Thermo Fisher Scientific, Inc, Waltham, MA

Table 2. Physicochemical and P-gp transport properties of the 9 compounds

Compound	MW	Class	pKa[1]	clogD$_{7.4}$	PSA[2]	Mdr1a/1b KO/WT
Carbamazepine	236	neutral	-	2.7	36	1.1[3]
Citalopram	324	basic	9.6	0.74	35	1.9[3]
Ganciclovir	255	neutral	-	-2.5	110	NA[4]
Metoclopramide	300	basic	9.6	0.18	58	6.6[3]
N-desmethylclozapine	313	basic	8.9	1.4	35	NA[4]
Quinidine	324	basic	9.3	1.6	41	36[3]
Risperidone	410	basic	8.4	2.2	57	10[3]
9-OH-Risperidone	426	basic	7.9	0.91	84	17[3]
Thiopental	242	acidic	7.8	2.8	50	1.2[3]

[1]calculated pKa

[2]polar surface area

[3]mdr1a/1b KO/WT ratios from Doran et al. (2005)

[4]no data available

Table 3. In vitro microdialysis probe recovery, unbound plasma and brain fraction, plasma, brain, and CSF concentration, and C_m of the 9 compounds in rats (Mean ± SD, n = 3-6)

Compound	Microdialysis Recovery (%)	f_{up}	f_{ub}	C_p (ng/ml)	C_b (ng/g)	C_{CSF} (ng/ml)	C_m (ng/ml)
Carbamazepine	55.2 ± 5.7	0.222 ± 0.024	0.0900 ± 0.0238	741 ± 114	884 ± 191	124 ± 72	172 ± 76
Citalopram	33.4 ± 0.6	0.355 ± 0.078	0.0285 ± 0.0046	392 ± 48	5290 ± 740	197 ± 19	219 ± 41
Ganciclovir	33.3 ± 5.7	0.972 ± 0.319	0.855 ± 0.546	1560 ± 220	207 ± 21	101 ± 35	111 ± 52
Metoclopramide	35.8 ± 1.8	0.635 ± 0.174	0.329 ± 0.209	251 ± 75	185 ± 51	42.4 ± 17	59 ± 9
N-desmethylclozapine	26.2 ± 3.1	0.0625 ± 0.0047	0.00569 ± 0.00030	325 ± 60	488 ± 70	4.92 ± 0	3.55 ± 2
Quinidine	29.4 ± 6.1	0.265 ± 0.015	0.0364± 0.0058	2570 ± 270	938 ± 99	249 ± 47	118 ± 73
Risperidone	18.3 ± 4.1	0.0798 ± 0.0046	0.0699± 0.0050	369 ± 66	95.9 ± 25.6	33.7 ± 8	15.6 ± 2
9-OH-Risperidone	47.8 ± 18.1	0.129 ± 0.011	0.0755± 0.0084	376 ± 116	33.2 ± 6	25.7 ± 12	5.37 ± 1
Thiopental	31.6 ± 5.6	0.111 ± 0.025	0.0986± 0.0124	48.6 ± 19	12.4 ± 3.9	3.22 ± 1	5.48 ± 2

All the concentrations were from the samples collected at 6 hour post start of infusion.

Table 4. The fold difference of use of the unbound brain concentration measured by brain homogenate method (C_{ub}), CSF concentration (C_{CSF}), and unbound plasma concentration (C_{up}) to predict the unbound brain interstitial concentration measured by brain microdialysis (C_m) of the 9 compounds

Compound	C_{ub} vs. C_m[1]	C_{CSF} vs. C_m[1]	C_{up} vs. C_m[1]
Carbamazepine	(2)	1	1
Citalopram	1	1	1
Ganciclovir	2	1	14
Metoclopramide	1	1	3
N-desmethylclozapine	1	1	6
Quinidine	(3)	2	6
Risperidone	(2)	2	2
9-OH-Risperidone	(2)	5	9
Thiopental	(4)	1	1

[1]Concentrations used in the calculations were the values at 6 hour post starting intravenous infusion.

The reported number represents the fold difference determined by the ratios of mean values rounded to the nearest whole number. Numbers in parentheses represent fold under-predictions.

Figure 1

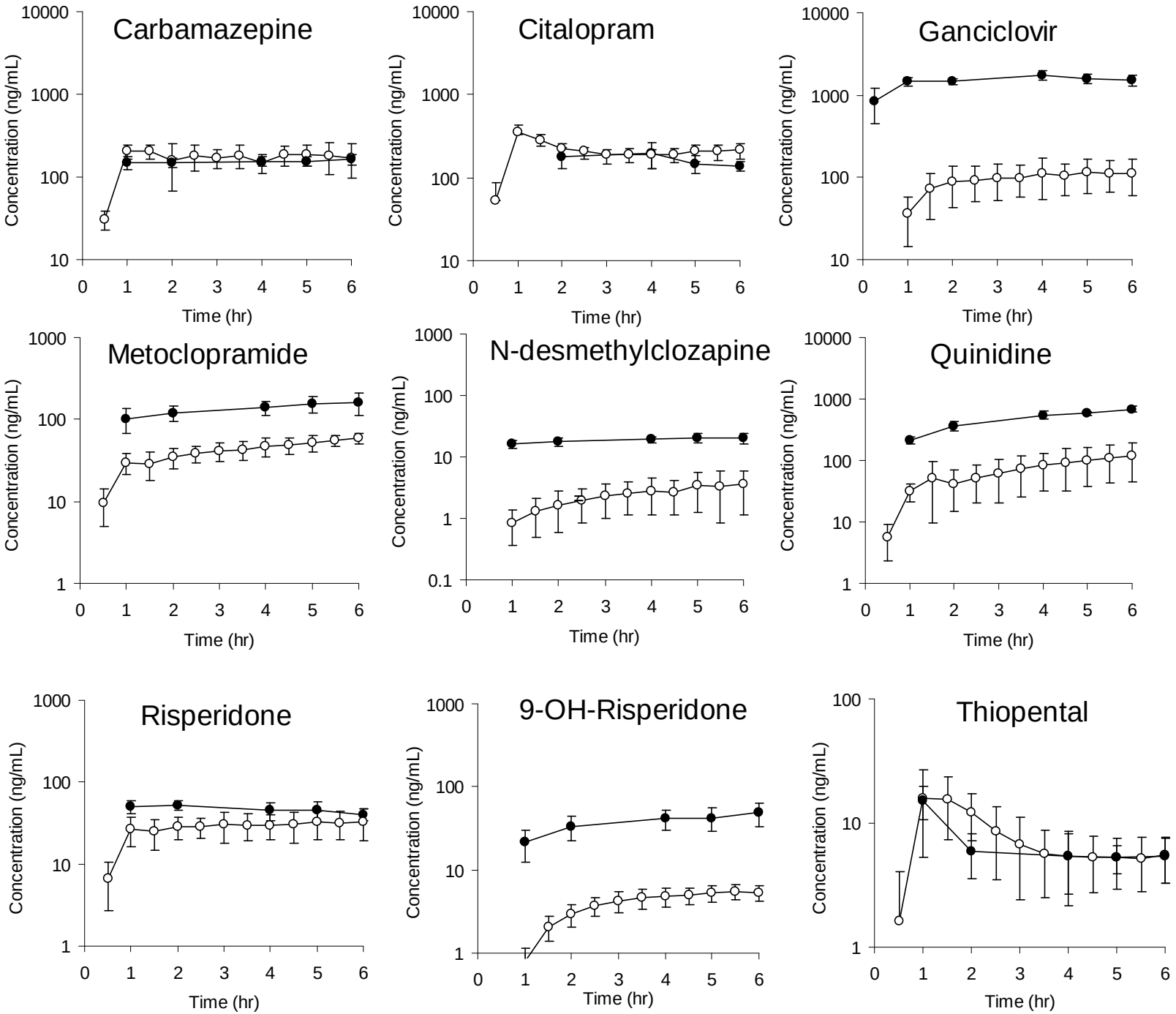

Figure 2

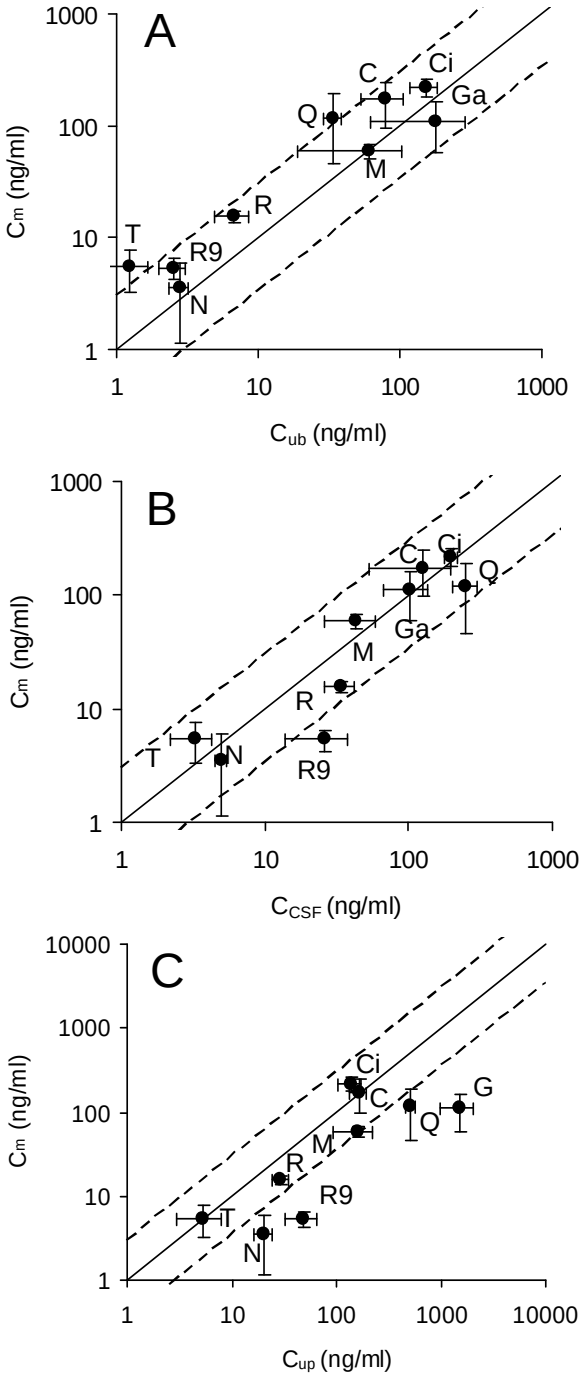

Figure 3