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EVALUATION OF CEREBROSPINAL FLUID CONCENTRATION AND PLASMA FREE CONCENTRATION AS A SURROGATE MEASUREMENT FOR BRAIN FREE CONCENTRATION

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Running title: CSF, plasma free and brain free concentration

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Abbreviations used are: BBB, blood-brain barrier; CSF, cerebrospinal fluid; BCSFB, blood CSF barrier; CNS, central nervous system; P-gp, P-glycoprotein; C_{CSF}, CSF drug concentration; $C_{u,plasma}$, plasma unbound drug concentration; $C_{u,brain}$, brain unbound drug concentration; AUC, area under the curve; PK/PD, pharmacokinetic/pharmacodynamic; NFPS, N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine; and CP-141938, methoxy-3-[(2-phenyl-piperadinyl-3-amino)-methyl]-phenyl-N-methyl-methane-sulfonamide.

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ABSTRACT

This study was designed to evaluate the use of cerebrospinal fluid (CSF) drug concentration and plasma unbound concentration ($C_{u,plasma}$) to predict brain unbound concentration ($C_{u,brain}$). The concentration-time profiles in CSF, plasma and brain of seven model compounds were determined following subcutaneous administration in rats. The $C_{u,brain}$ was estimated from the product of total brain concentrations and unbound fractions, which were determined using brain tissue slice and brain homogenate methods. For theobromine, theophylline, caffeine, fluoxetine and propranolol, which represent rapid brain penetration compounds with a simple diffusion mechanism, the ratios of the area under the curve (AUC) of $C_{u,brain}/C_{CSF}$ and $C_{u,brain}/C_{u,plasma}$ were 0.27-1.5 and 0.29-2.1, respectively, using brain slice method and were 0.27-2.9 and 0.36-3.9, respectively, using brain homogenate method. P-glycoprotein substrate, CP-141938, had $C_{u,brain}/C_{CSF}$ and $C_{u,brain}/C_{u,plasma}$ ratios of 0.57 and 0.066, respectively. The slow brain penetrating compound, NFPS, had $C_{u,brain}/C_{CSF}$ and $C_{u,brain}/C_{u,plasma}$ ratios of 0.94 and 0.12 using the brain slice method and 0.15 and 0.018 using the brain homogenate method, respectively. Therefore, for quick brain penetration with a simple diffusion mechanism compounds, C_{CSF} and $C_{u,plasma}$ represent $C_{u,brain}$ equally well; for efflux substrates or slow brain penetration compounds, C_{CSF} appears to be equivalent to or more accurate than $C_{u,plasma}$ to represent $C_{u,brain}$. Thus, we hypothesize that C_{CSF} is equivalent to or better than $C_{u,plasma}$ to predict $C_{u,brain}$. This hypothesis is supported by the literature data.

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It is a commonly accepted assumption that unbound or free drug is the species available for interaction with drug targets within the body and this is referred to as the free drug hypothesis. For drugs with an intended action in the CNS it is assumed that unbound drug in interstitial spaces in the brain ($C_{u,brain}$) is in direct contact or in equilibrium with the site of action (de Lange and Danhof, 2002). Therefore, in preclinical and clinical pharmacokinetic/pharmacodynamic (PK/PD) studies, it is critical to determine $C_{u,brain}$ for brain-targeted compounds. In preclinical PK/PD studies, in order to prove the mechanism of action or to validate an in vivo efficacy model, it is necessary to demonstrate the correlation between the affinity (e.g., K_i or IC_{50}) determined from in vitro pharmacology assays and the in vivo efficacious drug concentration at the site of action. Furthermore, in Phase I clinical trials, it is important to determine if a safe dose is observed that will result in sufficient $C_{u,brain}$ to demonstrate efficacy in Phase II trials. As the brain is separated from the systemic circulation by the blood-brain barrier (BBB) and the blood cerebrospinal fluid barrier (BCSFB), it has been a challenge to directly estimate $C_{u,brain}$. Microdialysis has been used to measure $C_{u,brain}$ but this method is resource-demanding and not applicable to compounds with particular physicochemical properties. Specifically, many compounds in the discovery stage of testing are often very lipophilic and it has been difficult to apply microdialysis to study these compounds due to high non-specific binding and poor recovery. (Carneheim and Stahle, 1991; Khramov and Stenken, 1999; Lindberger et al., 2002). In clinical trials, brain microdialysis cannot be readily used due to ethical reasons except in special circumstances (Scheyer et al., 1994a; Scheyer et al., 1994b; Joukhadar et al., 2001; Hillered et al., 2005). Other noninvasive imaging technologies, such as positron emission tomography, have been used to study mechanism of action, such as receptor occupancy in brain tissue. However, the imaging ligands for many novel drug targets may not be available or cannot be developed quickly for decision making during clinical trials (Cunningham et al., 2004).

Two surrogate approaches, namely plasma unbound concentration ($C_{u,plasma}$) and CSF concentration (C_{CSF}), have been used to estimate the $C_{u,brain}$ indirectly. Obviously, due to efflux transporters at the BBB, $C_{u,plasma}$ may not be equal to $C_{u,brain}$ (Liu and Chen, 2005). Data from brain microdialysis indicate that $C_{u,plasma}$ is higher than $C_{u,brain}$ for many of the compounds that have been studied (Hammarlund-Udenaes et al., 1997; Sawchuk and Elmquist, 2000). Because CSF is in direct contact with the brain tissue, it is assumed to readily equilibrate with brain interstitial fluid concentration (Meineke et al., 2002; Shen et al., 2004). CSF has been used as a common surrogate measure for $C_{u,brain}$ in clinical pharmacology studies (Bonati et al., 1982; Cherubin et al., 1989; Garver, 1989; Reiter and Doron, 1996; Ostermann et al, 2004). Nevertheless, the value of measuring C_{CSF} have been challenged (Bonati et al., 1982;

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de Lange and Danhof, 2002). Studies in the last decade using molecular biology approaches have demonstrated that the expression of many transporters at BCSFB is different from that at the BBB, supporting the opinion that the CSF drug concentration can significantly deviate from $C_{u,brain}$ (Kusuhara and Sugiyama, 2004).

Although numerous studies have been conducted to examine the relationship between C_{CSF}, $C_{u,plasma}$ and $C_{u,brain}$, no studies have been conducted to compare C_{CSF} and $C_{u,plasma}$ in prediction of $C_{u,brain}$. The objective of this work was to examine the accuracy of using C_{CSF} and $C_{u,plasma}$ to predict $C_{u,brain}$. It was recognized that generalizations like this could have serious pitfalls. However, we hoped to identify situations where these empirical observations are reasonable to facilitate the drug discovery and development process for CNS targeted agents.

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MATERIALS AND METHODS

Chemicals. Caffeine, fluoxetine, propranolol, theobromine, and theophylline were obtained from Sigma-Aldrich (St. Louis, MO). N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS) and methoxy-3-[(2-phenyl-piperadinyl-3-amino)-methyl]-phenyl-N-methyl-methane-sulfonamide (CP-141938) were synthesized at Pfizer Global Research and Development Laboratories (Groton, CT) with purity greater than 98%. All other chemicals used in the experiments were of the highest available grade.

Animal Experiments. Male Sprague-Dawley rats (250-280 g) were obtained from Charles River (Raleigh, NC). They were housed at controlled temperature and humidity in an alternating 12-hour light and dark cycle with free access to food and water. Rats received caffeine (10 mg/kg), CP-141938 (5 mg/kg), fluoxetine (10 mg/kg), NFPS (10 mg/kg), propranolol (5 mg/kg), theobromine (10 mg/kg), or theophylline (10 mg/kg) subcutaneously. The doses were prepared in 0.9% saline and delivered in a volume of 2 mL/kg. After the animals were sacrificed in a CO_2 chamber, approximately 50 µL CSF samples were collected at designated times between 10 minutes and 24 hours post dose via cisterna magna puncture and were stored at –20°C prior to analysis. In the same study, blood and brain samples were collected and the results were published previously (Liu et al., 2005).

Sample Analysis. Twenty µL of CSF and acetonitrile were mixed in silanized 96-well glass tubes. 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 3000 or 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 HPLC column. The HPLC-MS/MS methods of the seven model compounds in plasma and brain samples have been described previously (Liu et al., 2005). The same methods were used for the CSF samples. The low limit of quantitation for caffeine, CP-141938, fluoxetine, NFPS, propranolol, theobromine, and theophylline were 1.0, 0.50, 2.5, 0.20, 0.5, 50, and 50 ng/mL, respectively. The assay accuracy was between 80% and 120%.

Data Analysis. The plasma free concentrations were calculated from the product of the plasma unbound fraction and plasma concentration. The brain free concentrations were calculated from the product of the brain unbound fraction and brain concentration. The unbound fraction in plasma was determined using equilibrium dialysis. The unbound fraction in brain was determined using brain homogenate and brain tissue slice method as

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described previously (Becker and Liu, in press; Liu et al., 2005). Briefly, for brain homogenate method brain tissue was homogenized in two volumes of buffer. The brain homogenate was spiked with a compound and incubated at 37°C for 5 h in an equilibrium dialysis apparatus. The unbound fractions determined in the diluted brain tissue homogenates was corrected to yield an estimate of unbound fraction in the intact brain tissue. For brain slices method, brain slices of the cortex (400 μm) were prepared using a McIlwain Tissue Chopper. The brain slices and buffer were spiked with a compound and incubated at 37°C for 6 h.

The area under the curve from time zero to infinity ($AUC_{(0-\infty)}$) was calculated using WinNonlin™ (version 3.2, Pharsight Corporation, Mountain View, CA) as the sum of the area from zero to the last time point using the trapezoidal rule and the area from the last time point to infinity using the ratio of plasma concentration at the last time point and the slope of the terminal phase.

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RESULTS

The compounds selected for evaluation included a range of physicochemical properties, BBB permeability, brain disposition profiles and efflux transporter activity and this information was presented in our previous work (Liu et al. 2004, Liu et al. 2005). The P-gp transport activity, the BBB permeability (quantified as permeability-surface area product, PS), unbound fractions in plasma and brain tissue, the AUC of plasma, CSF and brain tissue concentrations, the AUC ratios of $C_{u,brain}/C_{CSF}$, $C_{u,brain}/C_{u,plasma}$, and $C_{CSF}/C_{u,plasma}$ are listed in Table 1. As shown in Table 1 the compounds selected represent weak or non-substrates as well as moderate to strong P-gp substrates.

Relationship between $C_{u,brain}$ and C_{CSF}: For theobromine, theophylline, caffeine, fluoxetine and propranolol, the time course of $C_{u,brain}/C_{CSF}$ increased over time and reached a plateau in less than 0.5 hour post dose for all compounds except fluoxetine, which reached a plateau 1 hour post dose (data not shown). The AUC ratios of $C_{u,brain}/C_{CSF}$ were between 0.27 and 1.5 using the brain unbound fraction determined from brain slices and between 0.27 and 2.9 using the brain unbound fraction determined from brain homogenate. For CP-141938, a P-gp substrate, its CSF concentrations equilibrated with brain concentrations in 0.5 hour. Its $C_{u,brain}/C_{CSF}$ ratios were 0.57 and 1.1 using the brain unbound fraction determined from brain slices and brain homogenate method, respectively. The weak P-gp substrate, NFPS, did not reach equilibrium between CSF and brain up to 24 hours post dose (data not shown). The NFPS AUC ratios of $C_{u,brain}/C_{CSF}$ were 0.94 and 0.15 using unbound fraction from brain slices and brain homogenate methods, respectively (Table 1). Thus, for the rapid brain penetration compounds that were studied, C_{CSF} is similar to $C_{u,brain}$. NFPS was the example of a slow brain penetration compound and in this case C_{CSF} was similar to $C_{u,brain}$ when based on the unbound fraction in brain tissue slices but C_{CSF} was greater than $C_{u,brain}$ when based on the unbound fraction obtained using the brain homogenate method (Figure 1).

Relationship between $C_{u,brain}$ and $C_{u,plasma}$: Like CSF, drug concentrations in plasma equilibrated rapidly with brain for theobromine, theophylline, caffeine, fluoxetine and propranolol (data not shown). The AUC ratios of $C_{u,brain}/C_{u,plasma}$ for the five compounds were between 0.29 and 2.1 using the brain unbound fraction determined from brain slices and between 0.36 and 3.9 using the brain unbound fraction determined from brain homogenate. The concentration of CP-141938 in plasma equilibrated with brain concentration by 0.5 hour. Its AUC ratios of $C_{u,brain}/C_{u,plasma}$ were 0.066 and 0.13 using the unbound fraction determined from brain slices and brain homogenate, respectively. For NFPS, a weak P-gp substrate, its $C_{u,plasma}$ did not equilibrate with brain concentrations up to 24 hours post dose. The $C_{u,brain}/C_{u,plasma}$ for NFPS was 0.12 using the brain unbound fraction determined from brain

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slices (Table 1). If the brain-unbound fraction determined from brain homogenate was used, the ratio was 0.018. These data indicate that the $C_{u,plasma}$ is close to $C_{u,brain}$ for all the compounds except CP-141938 and NFPS. The $C_{u,plasma}$ of CP-141938 and NFPS were much greater than their $C_{u,brain}$ (Figure 1)

Relationship between C_{CSF} and $C_{u,plasma}$: For all seven compounds, the CSF and plasma concentrations showed similar terminal half-lives (data not shown). The time-course of $C_{CSF}/C_{u,plasma}$ ratio increased over time and reached a plateau rapidly. For theobromine, theophylline, caffeine, fluoxetine, and propranolol the AUC ratios of $C_{CSF}/C_{u,plasma}$ were between 0.7 and 1.4. For CP-141938 and NFPS, their $C_{CSF}/C_{u,plasma}$ ratios were 0.11 and 0.13, respectively (Table 1).

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DISCUSSION

The seven model compounds were selected as their physicochemical properties, brain disposition, binding properties, and transport properties have been well characterized in our previous study (Liu et al., 2005). The unbound concentrations were considered similar if their values were within 3-fold. This criterion was chosen to allow for differences due to experimental error and for actual differences that would be considered to have significant pharmacological consequence (Maurer et al., 2005).

According to the results from this study, we hypothesize that although C_{CSF} may not necessarily equal to $C_{u,brain,}$ it is equivalent to or better than $C_{u,plasma}$ to predict $C_{u,brain}$. For five of the seven model compounds in the present study, their $C_{u,plasma}$ was within 3-fold of their $C_{u,brain}$. For the other two compounds, their $C_{u,plasma}$ was 15- to 56-fold of their $C_{u,brain}$. In contrast, the C_{CSF} was within 3-fold of the $C_{u,brain}$ for the seven compounds except NFPS. The C_{CSF} of NFPS was similar to the $C_{u,brain}$ using the unbound fraction determined with brain slice method and was 6.7-fold of the $C_{u,brain}$ using the unbound fraction determined with brain homogenate method. This discrepancy was due to the unbound fraction in brain slice was approximately seven-fold greater than the value measured using brain homogenate. The lower brain unbound fraction measured using the brain homogenate method was probably caused by greater accessing to the binding sites that are normally inaccessible to a compound in the intact brain tissue. This view is consistent with our previous data that using the brain slices method the predicted rat brain to plasma ratio was within 4-fold of the observed in vivo value but using brain homogenate method the predicted brain to plasma ratio was 27-fold greater than observed one (Becker and Liu, in press). The NFPS data from the present and previous studies indicate brain slice method is more accurate than brain homogenate method to determine brain unbound fraction. More studies are needed to confirm this observation.

Our hypothesis is supported by the data in the literature. Shen et al. (2004) compiled a data set generated from pre-clinical animals for 20 compounds, where the $C_{u,brain}$ was determined using microdialysis as the interstitial drug concentrations. Figure 2 supports that C_{CSF} is closer to $C_{u,brain}$ than $C_{u,plasma}$ for all the compounds except for morphine-6-glucuronide. The C_{CSF} of morphine-6-glucuronide is significantly greater (19-fold) than $C_{u,brain}$ but $C_{u,plasma}$ is similar to $C_{u,brain}$ (Stain-Texier et al., 1999). These results may be due to the CSF sink effect as morphine-6-glucuronide has low BBB permeability or different transporters at BBB and BCSFB. If this compound is excluded from the data set, the $C_{u,brain}/C_{CSF}$ and $C_{u,brain}/C_{u,plasma}$ values (mean ± SD) are 0.74 ± 0.58 and 0.26 ± 0.37 (n = 19), respectively. Our results are also consistent with a recent study in mice by Maurer et al (2005) where the brain

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unbound fraction was determined using the brain homogenate method. For 22 of 33 CNS drugs, their C_{CSF}, $C_{u,plasma}$ and $C_{u,brain}$ exhibited similar values. For four drugs, 9-hydroxyrisperidone, risperidone, sulpiride, and thiopental C_{CSF} is more accurate than $C_{u,plasma}$ in prediction of $C_{u,brain}$. Only two drugs, buspirone and caffeine, the C_{CSF} deviates significantly from $C_{u,brain}$ when compared to the corresponding $C_{u,plasma}$. However, caffeine in the present study clearly demonstrated identical concentrations for C_{CSF}, $C_{u,plasma}$ and $C_{u,brain}$ in rats.

It is interesting to note that the ratio of $C_{u,brain}/C_{CSF}$ is less than unity for most compounds in the present study and in the literature as well (Figure 2). As CSF protein concentrations are normally 0.5% or less of the respective plasma concentrations, it has been assumed that the protein binding in CSF is negligible in most CSF studies (Lin and Lu, 1997). This assumption may lead to an under-estimation of the true ratio of $C_{u,brain}/C_{CSF}$ for extensive protein binding compounds as the protein binding can be significant in CSF for lipophilic amines (Nyberg et al, 1981 and Wode-Helgodt and Alfredsson, 1981). Shen at al. (2004) has proposed that the unbound fraction of drug in CSF needs to be determined to more accurately assess drug concentrations at the biophase in CNS. Further studies are needed to examine whether the $C_{u,brain}/C_{CSF}$ ratios are closer to unity if the protein binding in the CSF is considered.

The seven model compounds can be empirically divided into two classes with the following characteristics:

Class I compounds: $C_{u,plasma} \approx C_{CSF} \approx C_{u,brain}$

Class II compounds: $C_{u,plasma} > C_{CSF} \geq C_{u,brain}$

Class I compounds included theobromine, theophylline, caffeine, fluoxetine and propranolol. Compounds in Class I penetrate the brain quickly and are presumed to cross the BBB and BCSFB via passive diffusion. Class II compounds include CP-141938 and NFPS. CP-141938 penetrates the brain quickly but is subject to significant P-gp efflux. Its brain to plasma ratio in mdr1a/b knockout mice was 50-fold of that in wild type mice (Smith et al., 2001). At equilibrium the C_{CSF} of CP-141938 is similar to its $C_{u,brain}$ but its $C_{u,plasma}$ is significantly higher than its $C_{u,brain}$. NFPS is a weak P-gp substrate and penetrates the brain slowly. Based on unbound fraction from brain slices, C_{CSF} predicts $C_{u,brain}$ and $C_{u,plasma}$ overpredicts $C_{u,brain}$; based on unbound fraction from brain homogenate, both C_{CSF} and $C_{u,plasma}$ over predict $C_{u,brain}$ but C_{CSF} is closer to $C_{u,brain}$ than $C_{u,plasma}$. We hypothesize that for Class I compounds, which penetrate the brain quickly via passive diffusion, C_{CSF} and $C_{u,plasma}$ show similar accuracy to predict $C_{u,brain}$; for Class II compounds, which penetrate the brain slowly or have high efflux activities, C_{CSF} appears to be similar to or better than $C_{u,plasma}$ to predict $C_{u,brain}$ although C_{CSF} may not equal to $C_{u,brain}$.

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Although only two Class II compounds were in the present study, our hypothesis is supported by the results from Maurer et al, (2005). In that study, three compounds, metocloperamide, risperidone, and 9-hydroxyrisperidone were shown as P-gp substrates with brain/plasma concentration ratios in the P-gp knockout vs. P-gp competent mice of 6.6, 10, and 17, respectively. Therefore, these compounds fit into Class II category. Based on our hypothesis, we would expect that C_{CSF} will equal to or be better than $C_{u,plasma}$ to predict $C_{u,brain}$ for these compounds. Indeed, this prediction is consistent with the observed data. We calculated the $C_{u,brain}/C_{u,plasma}$ and $C_{u,brain}/C_{CSF}$ ratios based on the total concentration ratios and binding in plasma and brain tissue homogenate. The $C_{u,brain}/C_{u,plasma}$ are 0.52, 0.26, and 0.02, and $C_{u,brain}/C_{CSF}$ ratios are 0.86, 2.7 and 0.12, respectively, in the P-gp competent mice. These data demonstrated that the C_{CSF} concentration is similar to the $C_{u,plasma}$ to predict $C_{u,brain}$ for metochloperamide and risperidone but better than $C_{u,plasma}$ for 9-hydroxyrisperidone although its C_{CSF} still over predicts the $C_{u,brain}$. Furthermore, we anticipate the advantage of using C_{CSF} to predict $C_{u,brain}$ for P-gp substrates in P-gp competent mice will be diminished in P-gp knockout mice as these P-gp substrates become Class I compounds in P-gp knockout mice. As expected, $C_{u,brain}/C_{u,plasma}$ ratios (3.5, 2.6, and 0.26) were similar to these of $C_{u,brain}/C_{CSF}$ (2.6, 4.1, and 0.20) for metocloperamide, risperidone, and 9-hydroxyrisperidone, respectively.

Our hypothesis implies that P-gp has a more significant impact on the $C_{u,brain}/C_{u,plasma}$ than on $C_{u,brain}/C_{CSF}$ ratios. This prediction is in good agreement with the data reported by Doran et al. (2005). The brain/plasma concentration ratios of P-gp substrates loperamide, verapamil, and quinidine in the P-gp knockout mice vs. P-gp competent mice were 9.3, 17, and 36, respectively, which were significantly greater than three-fold. However, their Brain/CSF concentration ratios in the P-gp knockout vs. P-gp competent mice were 1.5, 1.9, and 3.6, which were within or close to three-fold.

Regardless a compound belongs to Class I or II, C_{CSF} may be used as a surrogate estimate for $C_{u,brain}$ in drug discovery setting as CSF samples can be readily collected during in vivo pharmacology screens from rodents and only single analytical assay is needed to estimate $C_{u,brain}$. For using $C_{u,plasma}$ to predict $C_{u,brain}$, two assays, total plasma concentration and protein binding, are required to estimate brain unbound concentration. Unlike animal studies, in clinical trials CSF collection adds substantial cost and increases the complexity of study design. For a Class I compound that has been characterized in pre-clinical study, if no in vitro data suggest it is a BBB efflux transporter substrate, C_{CSF} may provide no additional information than $C_{u,plasma}$. For a Class II compound, it may be valuable to determine C_{CSF} as it may more closely represent $C_{u,brain}$ than $C_{u,plasma}$. By comparing the relationship between C_{CSF}

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and $C_{u,plasma}$ in humans and the relationship among C_{CSF}, $C_{u,plasma}$ and $C_{u,brain}$ in animals, we may be able to estimate $C_{u,brain}$ in humans assuming no significant species difference in CNS drug disposition. Other approaches, such as imaging analysis, may be needed to verify the estimation. This view is consistent with Shen's opinions (2004) that CSF penetration studies in animals can serve as predictive models in human drug development even for compounds that are substrates of transporters at the BBB and BCSFB. This proposed Classification system according to the characteristics of drug disposition in CNS was based on a limited number of compounds and should be considered as a hypothesis. More studies are needed to further confirm this hypothesis.

In summary, the results in the present study indicate that although C_{CSF} is equivalent to $C_{u,plasma}$ as a surrogate measurement for $C_{u,brain}$ for rapid brain penetration compounds that across BBB and BCSFB by passive diffusion. C_{CSF} is more predictive than $C_{u,plasma}$ for efflux transporter substrates or for slow brain penetrating compounds. It is valuable to determine C_{CSF} in drug discovery and possibly in some circumstances in clinical drug development

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References

Becker SL and Liu X Evaluation of the utility of brain slice methods to study brain penetration. *Drug Metab Dispos*. in press.

Bonati M, Kanto J and Tognoni G (1982) Clinical pharmacokinetics of cerebrospinal fluid. *Clin Pharmacokin* **7**:312-335.

Carneheim S and Stahle L (1991) Microdialysis of lipophilic compounds: a methodological study. *Pharmacol Toxicol* **69**:378

Cherubin CE, Eng RH, Norrby R, Modai J, Humbert G, and Overturf G (1989) Penetration of newer cephalosporins into cerebrospinal fluid. Reviews of infectious diseases 11:526-548.

Cunningham VJ, Gunn RN and Matthews JC (2004) Quantification in positron emission tomography for research in pharmacology and drug development. *Nuclear Med Comm* **25**:643-646.

de Lange EC and Danhof M (2002) Considerations in the use of cerebrospinal fluid pharmacokinetics to predict brain target concentrations in the clinical setting: implications of the barriers between blood and brain. *Clin Pharmacokinet* **41**:691-703.

Doran A, Obach RS, Smith BJ, Hosea NA, Becker S, Callegari E, Chen C, Chen X, Choo E, Cianfrogna J, Cox LM, Gibbs JP, Gibbs MA, Hatch H, Hop CECA, Kasman IN, LaPerle J, Liu J, Liu X, Logman M, Maclin D, Nedza FM, Nelson F, Olson E, Rahematpura S, Raunig D, Rogers S, Schmidt K, Spracklin DK, Szewc M, Troutman M, Tseng E, Tu M, Van Deusen JW, Venkatakrishnan K, Walens G, Wang EQ, Wong D, Yasgar AS and Zhang C (2005) The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: Evaluation using the Mdr1a/1b knockout mouse model. *Drug Metab Dispos* **33:**165-174.

Garver DL (1989) Neuroleptic drug levels and antipsychotic effects: a difficult correlation; potential advantage of free (or derivative) versus total plasma levels. *J Clin Psychopharmacol* **9**:277-281.

Hammarlund-Udenaes M, Paalzow LK and de Lange EC (1997) Drug equilibration across the blood-brain barrier--pharmacokinetic considerations based on the microdialysis method. *Pharm Res* **14**:128-134.

Hillered L, Vespa PM and Hovda DA (2005) Translational neurochemical research in acute human brain injury: the current status and potential future for cerebral microdialysis. *J Nutr* **22**:3-41.

Joukhadar C, Derendorf H and Muller M (2001) Microdialysis. A novel tool for clinical studies of anti-infective agents. *Eur J Clin Pharmacol* **57**:211-219.

Khramov AN and Stenken JA (1999) Enhanced microdialysis extration efficiency of ibuprofen in vitro by facilitated transport with Beta-cyclodextrin. *Anal Chem* **71**:1257-1264.

Kusuhara H and Sugiyama Y (2004) Efflux transport systems for organic anions and cations at the blood-CSF barrier. *Adv Drug Delivery Rev* **56**:1741-1763.

Lin JH and Lu AYH (1997) Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol. Rev* **49:**403–449.

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Lindberger M, Tomson T and Stahle L (2002) Microdailysis sampling of carbamazepine, phenytoin and phenobarbital in subcutaneous extracellular fluid and subdural cerebrospinal fluid in humans: an in vitro and in vivo study of adsorption to the sampling device. *Pharmacol Toxicol* **91**:158-165.

Liu X, Tu M, Kelly RS, Chen C and Smith BJ (2004) Development of a computational approach to predict blood-brain barrier permeability. Drug Metab Dispos 32:132-139.

Liu X and Chen C (2005) Strategies to optimize brain penetration in drug discovery. *Curr Opin Drug Disc Dev* **8**:505-512.

Liu X, Smith BJ, Chen C, Callegari E, Becker SL, Chen X, Cianfrogna J, Doran AC, Doran SD, Gibbs JP, Hosea N, Liu J, Nelson FR, Szewc MA and Van Deusen J (2005) Use of a physiologically based pharmacokinetic model to study the time to reach brain equilibrium: An experimental analysis of the role of blood-brain barrier permeability, plasma protein binding, and brain tissue binding. *J Pharmacol Exp Ther* **313**:1254-1262.

Maurer TS, DeBartolo DB, Tess DA and Scott DO (2005) Relationship between exposure and nonspecific binding of thirty-three central nervous system drugs in mice. *Drug Metab Dispos* **33**:175-181.

Meineke I, Freudenthaler S, Hofmann U, Schaeffeler E, Mikus G, Schwab M, Prange HW, Gleiter CH and Brockmoller J (2002) Pharmacokinetic modelling of morphine, morphine-3-glucuronide and morphine-6-glucuronide in plasma and cerebrospinal fluid of neurosurgical patients after short-term infusion of morphine. *Br J Clin Pharmacol* **54:**592-603.

Nyberg G, Axelsson R and Martensson E (1981) Cerebrospinal fluid concentrations of thioridazine and its main metabolites in psychiatric patients, Eur. J. Clin. Pharmacol. 19: 139– 148.

Ostermann, S, Csajka C, Buclin T, Leyvraz S, Lejeune F, Decosterd LA, and Stupp R (2004) Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients. *Clin Cancer Res* **10**:3728–3736.

Reiter PD and Doron MW (1996) Vancomycin cerebrospinal fluid concentrations after intravenous administration in premature infants. *J perinatology* 16:331-335.

Sawchuk RJ and Elmquist WF (2000) Microdialysis in the study of drug transporters in the CNS. *Adv Drug Delivery Rev* **45**:295-307.

Scheyer RD, During MJ, Hochholzer JM, Spencer DD, Cramer JA and Mattson RH (1994a) Phenytoin concentrations in the human brain: an in vivo microdialysis study. *Epilepsy Res* **18**:227-232.

Scheyer RD, During MJ, Spencer DD, Cramer JA and Mattson RH (1994b) Measurement of carbamazepine and carbamazepine epoxide in the human brain using in vivo microdialysis. *Neurology* **44**:1469-1472.

Shen DD, Artru AA and Adkison KK (2004) Principles and applicability of CSF sampling for the assessment of CNS drug delivery and pharmacodynamics. *Adv Drug Delivery Rev* **56**:1825-1857.

Smith BJ, Doran AC, McLean S, Tingley FDr, O'Neil BT and Kajiji SM (2001) P-glycoprotein efflux at the blood-brain barrier mediates differences in brain disposition and pharmacodynamics between two structurally related neurokinin-1 receptor antagonists. *J Pharmacol Exp Ther* **298**:1252-1259.

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DMD Fast Forward. Published on June 7, 2006 as DOI: 10.1124/dmd.105.008201
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Stain-Texier F, Boschi G, Sandouk P and Scherrmann JM (1999) Elevated concentrations of morphine 6-beta-D-glucuronide in brain extracellular fluid despite low blood-brain barrier permeability. *Br J Pharmacol* **128**:917-924.

Wode-Helgodt B and Alfredsson G (1981) Concentrations of chlorpromazine and two of its active metabolites in plasma and cerebrospinal fluid of psychotic patients treated with fixed drug doses, Psychopharmacology 73: 55– 62.

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FIGURE LEGENDS

Figure 1. AUC ratio of $C_{u,brain}/C_{u,plasma}$ (solid bars) and $C_{u,brain}/C_{CSF}$ (open bars) of seven model compounds in rats. The data were from Table 1. The solid and broken lines represent unity and 3-fold boundaries, respectively. The brain unbound fractions in A and B were determined using the brain slice method and homogenate method, respectively.

Figure 2. The plot of $C_{u,brain}/C_{u,plasma}$ (solid bars) and $C_{u,brain}/C_{CSF}$ (open bars) of 20 compounds in rats or rabbits. $C_{u,brain}$ represents brain interstitial drug concentrations determined using microdialysis. The concentrations were the values observed at steady state or as AUC after single administration. The data were modified from Shen et al. (2004). The solid and broken lines represent unity and 3-fold boundaries, respectively.

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Table 1. P-gp activities, unbound fractions, AUC, and AUC ratios of seven model compounds.

	Class I					Class II	
	Theobromine	Theophylline	Caffeine	Fluoxetine	Propranolol	CP-141938	NFPS
BP_{ko}/BP_{wt}[a]	1.8[d]	1.3[d]	1.1[g]	1.5[g]	1.5[d]	50[h]	2.1[d]
In Situ PS[b] (mL/hr/kg)	23	31	223	619	1043	6.4	31
Plasma Unbound Fraction[c]	0.92	0.29	0.96	0.060	0.15	0.56	0.041
Brain Unbound Fraction[d] (Slice)	0.62	0.31	0.71	0.0027	0.019	0.11	0.011
Brain Unbound Fraction[c] (Homogenate)	0.61	0.39	1.1	0.00094	0.036	0.22	0.0017
$AUC_{(Plasma, 0-\infty)}$ (ng•hr/mL)	4670	6990	3250	195	237	296	1480
$AUC_{(CSF, 0-\infty)}$ (ng•hr/mL)	5790	1420	3140	13.3	48.0	18.9	7.59
$AUC_{(Brain, 0-\infty)}$ (ng•hr/mL)	2540	1910	2010	5140	3880	98.8	648
$C_{u,brain}/C_{CSF}$[e] (Slice)	0.27	0.42	0.45	1.0	1.5	0.57	0.94
$C_{u,brain}/C_{u,plasma}$[e] (Slice)	0.37	0.29	0.46	1.2	2.1	0.066	0.12
$C_{u,brain}/C_{CSF}$[f] (Homogenate)	0.27	0.52	0.70	0.36	2.9	1.1	0.15
$C_{u,brain}/C_{u,plasma}$[f] (Homogenate)	0.36	0.37	0.71	0.41	3.9	0.13	0.018
$C_{CSF}/C_{u,plasma}$	1.3	0.70	1.0	1.1	1.4	0.11	0.13

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[a]BP_{ko} and BP_{ko} represents the brain-to-plasma concentration ratio in mdr1a/1b gene knockout and FVB mice, respectively.

[b]Liu et al. (2004): BBB permeability-surface area product, PS

[c]Liu et al. (2005): unbound fraction in plasma determined using equilibrium dialysis

[d]Becker and Liu (in press): unbound fraction in brain tissue determined using brain slices

[e]$C_{u,brain}$: the brain unbound fraction was determined from brain slice method.

[f]$C_{u,brain}$: the brain unbound fraction was determined from brain homogenate method.

[g]Doran et al (2005)

[h]Smith et al (2001)

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Figure 1

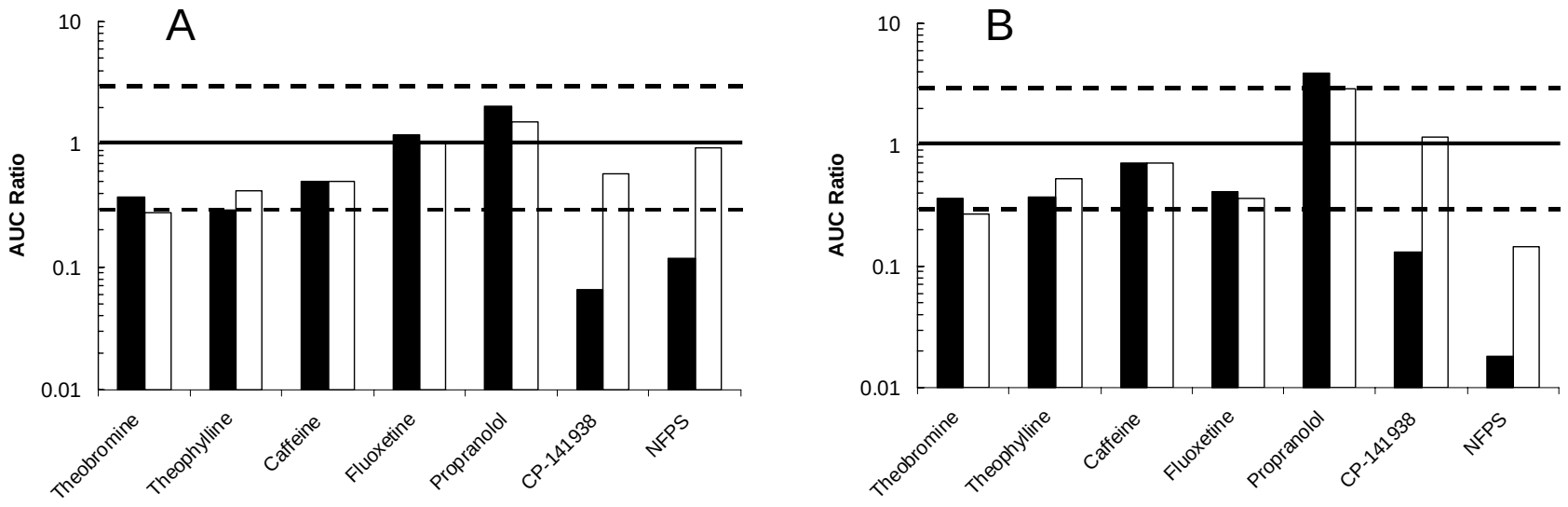

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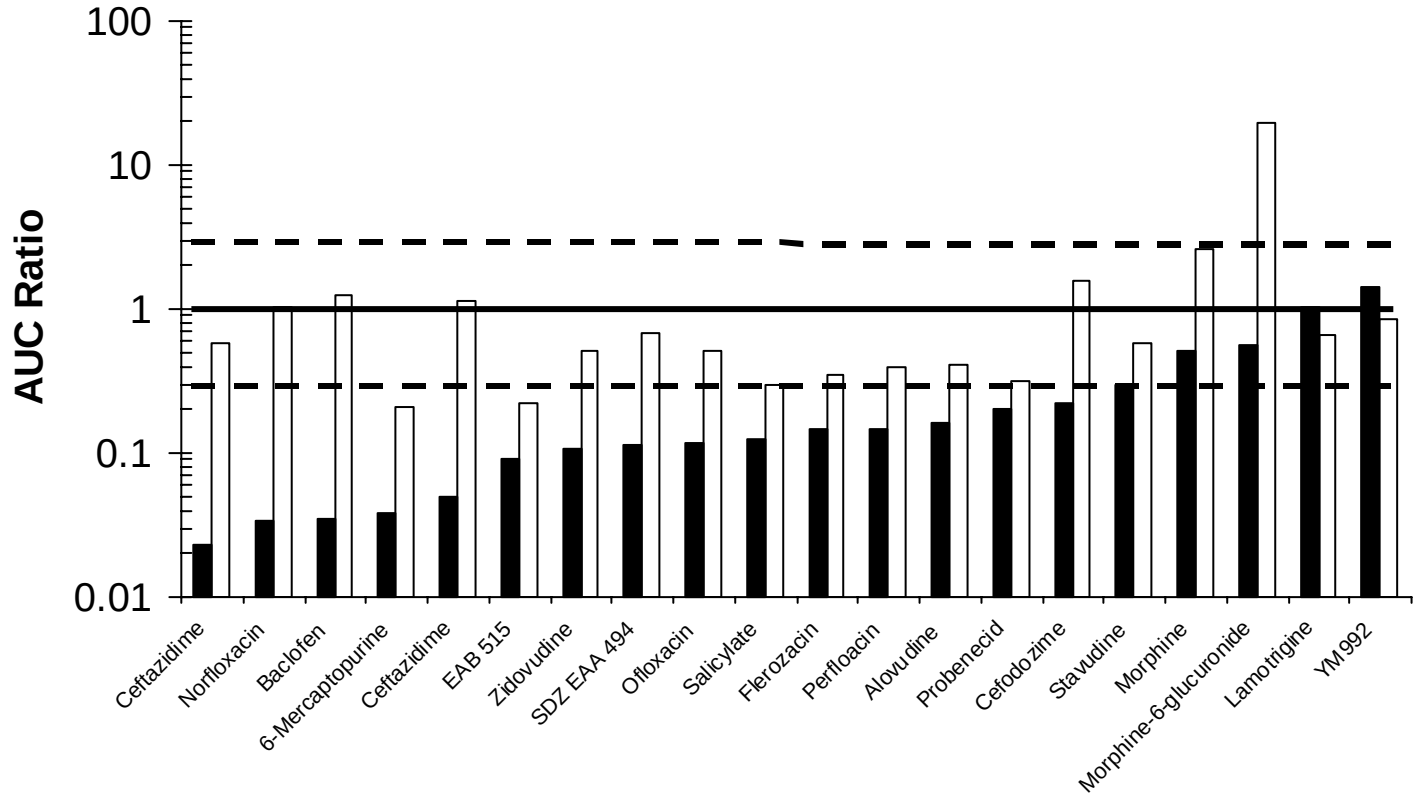
Figure 2



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