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# Utility of Cerebrospinal Fluid Drug Concentration as a Surrogate for Unbound Brain Concentration in Nonhuman Primates

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**Summary:** In central nervous system drug discovery, cerebrospinal fluid (CSF) drug concentration ($C_{CSF}$) has been widely used as a surrogate for unbound brain concentrations ($C_{u,brain}$). However, previous rodent studies demonstrated that when drugs undergo active efflux by transporters, such as P-glycoprotein (P-gp), at the blood-brain barrier, the $C_{CSF}$ overestimates the corresponding $C_{u,brain}$. To investigate the utility of $C_{CSF}$ as a surrogate for interstitial fluid (ISF) concentration ($C_{ISF}$) in nonhuman primates, this study simultaneously determined the $C_{CSF}$ and $C_{ISF}$ of 12 compounds, including P-gp substrates, under steady-state conditions in cynomolgus monkeys using intracerebral microdialysis coupled with cisternal CSF sampling. Unbound plasma concentrations of non- or weak P-gp substrates were within 2.2-fold of the $C_{ISF}$ or $C_{CSF}$, whereas typical P-gp substrates (risperidone, verapamil, desloratadine, and quinidine) showed ISF-to-plasma unbound ($K_{p,uu,ISF}$) and CSF-to-plasma unbound concentration ratios ($K_{p,uu,CSF}$) that were appreciably lower than unity. Although the $K_{p,uu,CSF}$ of quinidine, verapamil, and desloratadine showed a trend of overestimating the $K_{p,uu,ISF}$, $K_{p,uu,CSF}$ showed a good agreement with $K_{p,uu,ISF}$ within 3-fold variations for all compounds examined. $C_{u,brain}$ of some basic compounds, as determined using brain homogenates, overestimated the $C_{ISF}$ and $C_{CSF}$. Therefore, $C_{CSF}$ could be used as a surrogate for $C_{ISF}$ in nonhuman primates.

**Keywords: P-glycoprotein; central nervous system; cerebrospinal fluid; interstitial fluid; nonhuman primates; microdialysis; drug discovery**

## Introduction

In drug discovery, the plasma concentration of unbound drugs ($C_{u,plasma}$) has been used to elucidate the correlation between pharmacokinetics and pharmacodynamics on the basis of the "free drug hypothesis".[1)] However, differences between the $C_{u,plasma}$ and the unbound brain concentration ($C_{u,brain}$) have been observed, mainly due to the restrictive role of the blood–brain barrier (BBB), which has highly developed tight junctions between adjacent endothelial cells and active efflux transporters, such as P-glycoprotein (P-gp/*ABCB1*),[2)] breast cancer resistance protein (BCRP/*ABCG2*),[3,4)] and multidrug resistance-associated protein 4 (MRP4/*ABCC4*),[5,6)] limiting drug penetration of the central nervous system (CNS). As only unbound drugs in the brain interstitial fluid (ISF) are available to interact with their targets in the CNS, a comparison of drug ISF concentrations ($C_{ISF}$) with the pharmacologically effective concentration estimated from *in vitro* pharmacological studies is crucial to quantitatively assess the mechanisms of action. Moreover, poor CNS exposure requires higher doses of a drug candidate to elicit an efficacious response in the CNS, resulting in higher drug exposure and a narrower therapeutic index against peripheral toxicity. Therefore, lead optimization with an appropriate approach to estimate $C_{ISF}$ of drug candidates is indispensable to CNS drug discovery.

Several approaches have been utilized to measure or estimate $C_{ISF}$, and microdialysis is the only method that can directly access brain ISF, in which the microdialysis probe is implanted in the brain to supply and collect the perfusate in equilibrium with drugs in the ISF.[7,8)] Due to technical challenges and the low throughput, it is difficult to routinely implement microdialysis in drug discovery. Instead, cerebrospinal fluid (CSF) drug concentration ($C_{CSF}$) has been widely used as a surrogate for $C_{ISF}$ on the assumption of rapid equilibrium between the ISF and CSF compartments.[9–12)] However, as expected from the complexity of the CSF anatomy and physiology, there have been cases where the $C_{CSF}$ deviated from the $C_{ISF}$;[13)] hence, it is important to consider the applicability and

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limitations of $C_{CSF}$ as a surrogate for $C_{ISF}$ for successful CNS drug discovery.

Several reports have demonstrated that the $C_{CSF}$-to-$C_{ISF}$ gradient is evident for substrates of the active efflux transporters P-gp and/or BCRP in rodents.[14–17] They are expressed not only at the BBB, but also in the choroid plexus epithelial cells constituting the blood–CSF barrier (BCSFB), where P-gp is reportedly localized to the cytoplasm or subapical membrane[18] and BCRP to the brush border membrane,[15] suggesting that they do not limit drug penetration into the CSF at the BCSFB. In addition, knockout of P-gp and/or BCRP diminished the gradient.[17] These findings support the supposition that CNS-to-blood directed active efflux by P-gp and/or BCRP is dominant at the BBB over the BCSFB and is a determinant of the $C_{CSF}$-to-$C_{ISF}$ gradient in the brain. However, these findings have been reported in rodents; therefore, the impact of active efflux on the gradient remains to be elucidated in nonhuman primates and humans.

Since nonhuman primates are considered to be well suited to investigate pharmacological targets in the CNS due to the high genetic similarity to humans, a detailed understanding of the CNS drug distribution contributes to CNS drug discovery. Although drug CNS distribution has been investigated in nonhuman primates using invasive or noninvasive methods,[19–22] little direct comparison of $C_{ISF}$ and $C_{CSF}$ has been carried out, particularly for P-gp substrates. The brain distribution of positron emission tomography radioligands and quantification of P-gp protein expression suggest the presence of interspecies differences in active efflux at the BBB between rodents and nonhuman primates or humans,[22–25] which also warrants further investigation in nonhuman primates.

Therefore, this study simultaneously determined and compared the $C_{ISF}$, $C_{CSF}$, and $C_{u,plasma}$ of 12 compounds, including P-gp substrates, under steady-state conditions using intracerebral microdialysis coupled with cisternal CSF sampling in cynomolgus monkeys. Moreover, unbound drug concentrations in brain tissues were determined by the brain homogenate method and compared with $C_{ISF}$ and $C_{CSF}$.

## Materials and Methods

**Chemicals:** Antipyrine, carbamazepine, lucifer yellow CH dilithium salt, metoclopramide hydrochloride, ondansetron hydrochloride dihydrate, propranolol hydrochloride, quinidine hydrochloride, and verapamil hydrochloride were purchased from Sigma Aldrich (St. Louis, MO). Midazolam, desloratadine, and risperidone were purchased from Wako Pure Chemical Industries (Osaka, Japan), LKT Laboratories (St Paul, MN), and AK Scientific (Union City, CA), respectively. A proprietary compound, E2074, synthesized in Tsukuba Research Laboratories, Eisai (Ibaraki, Japan) was used.[26] All other reagents and solvents were of analytical grade and commercially available.

**Animals:** All experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee of Trans Genic (Kumamoto, Japan) and Eisai. Eight male cynomolgus monkeys (4–7 years old, 3.6–5.1 kg) supplied by Trans Genic were used for *in vivo* microdialysis studies. All surgeries and procedures were performed by veterinarians in Trans Genic, and animal condition before, during, and after surgery was closely monitored by the veterinarians. All efforts were made to minimize suffering.

**Intracerebral microdialysis with CSF and blood sampling:** One to 2 weeks before the initial dosing, animals were pretreated with an analgesic (meloxicam, 0.3 mg/kg, s.c.), and a heparin-coated polyurethane catheter (Solomon Scientific, San Antonio, TX) was surgically placed into the cisterna magna under ketamine (10 mg/kg, i.m.) and pentobarbital anesthesia (30 mg/kg, i.v.). Cefamezin (100 mg/body, i.v.) was administered once daily for three days post-operatively. Thereafter, under anesthesia, 1-mm burr holes were created at approximately 10 mm from the midline in the right and left anterior parietal bones, and a small dural incision was made. A CG-8 guide cannula (Eicom, Kyoto, Japan) with AD-8 dummy cannula (Eicom) was implanted in the cerebral cortex and fixed to the skull, and the surgical site was treated with cefamezin solution. On the day of dosing, a C-I-8 microdialysis probe (Eicom) with a 5-mm regenerated cellulose membrane (50-kDa molecular weight cut off) was inserted into the guide cannula under the anesthesia, so that the probe membrane tip was placed at 10 mm from the brain surface. A second microdialysis probe was also implanted in the contralateral side in the same manner for retrodialysis. After full recovery of the animals from the anesthesia and completion of retrodialysis study, the microdialysis probe was perfused with Ringer HEPES buffer (RHB) (147 mM NaCl, 4.7 mM KCl, 0.6 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $CaCl_2 \cdot 2H_2O$, 5 mM HEPES, pH 7.4) at a flow rate of 1.5 μL/min using a syringe pump (Harvard Apparatus, Holliston, MA). One to 2 h after the preperfusion, a cocktail of two or three compounds was infused *via* the cephalic vein following an intravenous bolus dose for antipyrine (infusion rate, 0.10 mg/h/kg; bolus dose, 0.23 mg/kg), carbamazepine (0.10; 0.22), desloratadine (0.30; 3.9), lamotrigine (0.020; 0.37), metoclopramide (0.30; 0.37), midazolam (0.10; 0.13), ondansetron (0.40; 0.81), propranolol (0.15; 0.68), quinidine (1.0; 1.6), risperidone (0.40; 0.51), verapamil (0.50; 1.2), E2074 (0.070; 0.19), diphenhydramine (0.30; 0.55), and ketotifen (1.0; 2.6). Although diphenhydramine and ketotifen were also evaluated, the results will be reported separately for other purposes. In the present study, cassette dosing was adopted to minimize the study period and the number of animals used. Since some compounds used in this study are reported to be P-gp inhibitors (*e.g.*, verapamil and quinidine), the dose levels were optimized so that the $C_{u,plasma}$ was kept equal to or less than lower limit of reported half maximal inhibitory concentrations for P-gp[27] based on a previous report.[28] The microdialysis dialysate was serially collected at 30-minute intervals up to 4 h postdose. Blood (0.5 mL per sampling point) and CSF (0.25 mL per sampling point) were also serially collected at 1-h intervals up to 4 h postdose *via* the saphenous vein and cisterna magna, respectively, and the blood samples were centrifuged to prepare plasma. The animals received a cocktail of two or three compounds on a once-weekly basis.

**Brain tissue sampling:** At least a one-week washout period was introduced after the microdialysis experimental phase. Then, each animal received a cocktail of two or three compounds (monkey-1, antipyrine, midazolam, and quinidine; monkey-2, carbamazepine, diphenhydramine, and verapamil; monkey-3, metoclopramide, ketotifen, and ondansetron; monkey-4, desloratadine, E2074, and risperidone; monkey-5, lamotrigine and propranolol) by intravenous infusion following an intravenous bolus dose as described above. At 4 h after infusion, the animals were euthanized by exsanguination under anesthesia, and the blood and brain tissues were collected. The brain tissues were sampled from six different regions of the cerebral cortex to confirm no regional difference in drug concentration within the cerebral cortex. The blood samples were centrifuged to prepare plasma. The