The Simultaneous Estimation of the Influx and Efflux Blood-Brain Barrier Permeabilities of Gabapentin Using a Microdialysis-Pharmacokinetic Approach

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Purpose. To determine the apparent bidirectional permeabilities of gabapentin (GBP) across the blood-brain barrier (BBB) using a novel microdialysis-pharmacokinetic approach.

Methods. Rats were administered intravenous infusions of [^{14}C]GBP to achieve clinically relevant steady-state plasma concentrations. Microdialysis was used to monitor GBP concentration in brain extracellular fluid (ECF) in conscious animals. Brain tissue GBP concentration was measured at termination. The BBB influx (CL_1) and efflux (CL_2) permeabilities of GBP were estimated with a hybrid pharmacokinetic model assuming that transport between intra-and extracellular space was more rapid than transport across the BBB. The time course of GBP concentration in brain tissue was determined independently to validate the model assumption.

Results and Conclusions. Simulations of the concentration-time course of GBP in brain tissue based on this modeling correlated well with the time-course of brain tissue concentrations determined after intravenous bolus administration and validated this pharmacokinetic-microdialysis approach for estimation of BBB permeabilities. The values for CL_1 and CL_2 were 0.042 (0.017) and 0.36 (0.16) ml/min·g-brain, respectively, indicating that GBP was more efficiently transported from brain ECF to plasma. The total brain tissue concentration of GBP was significantly higher than the ECF concentration at steady-state due to intracellular accumulation and tissue binding, that if not considered, will lead to underestimated efflux BBB permeability using the tissue homogenate-pharmacokinetic approach.

KEY WORDS: microdialysis; gabapentin; retrodialysis; blood-brain barrier; transport; clearance; permeability; rat.

INTRODUCTION

Quantitative brain microdialysis has been made possible as new calibration techniques have been developed over the past several years (1,2). However, the information derived from brain microdialysis is still largely reported in qualitative terms, i.e., the half-life of drug in brain extracellular fluid (ECF) is compared to the plasma half-life or to the time course of pharmacologic effect. Microdialysis monitors the free concentration of drug in ECF, one of the driving forces for transport across the BBB, and therefore it is theoretically possible to estimate both the influx and efflux blood-brain barrier (BBB) permeabilities of a compound using this technique. Recently, the influx and efflux permeabilities of zidovudine were estimated using a brain microdialysis-pharmacokinetic approach (3). This analysis was achieved by assuming that clearance between intra- and extracellular space was much greater than clearance across the BBB. In the present study, the influx and efflux BBB permeabilities of the anticonvulsant, gabapentin (1-(aminomethyl) cyclohexaneacetic acid; Neurontin®; GBP), were estimated using a similar approach. Further, the model assumption was validated using the conventional brain tissue homogenate-pharmacokinetic analysis method (4).

MATERIALS AND METHODS

Materials

[^{14}C]GBP, specific activity 24.9 μCi/mg, 98.61% radiochemical purity, 85.9% chemical purity was synthesized by the Radiochemical Section, Parke-Davis Pharmaceutical Research Division. Microdialysis probes (CMA/12; Carnegie Medicine, Acton, MA) 4-mm membrane length, were used. Artificial CSF solution was freshly prepared and filtered (0.2 μm) before use (5). A [^{14}C]GBP solution for intravenous injection (2.0 mg/ml, activity 49.8 μCi/mL) was prepared in 0.9% saline and filtered before administration. An aliquot of this solution was diluted with CSF to yield a final concentration of 0.4 μg/ml (2.2×10^4 dpm/mL) for *in vitro* microdialysis and *in vivo* retrodialysis recovery calibration experiments.

Surgical Preparation for Microdialysis Experiment

Animal studies complied with the Principles of Laboratory Animal Care guidelines (NIH publication #85-23, revised 1985). Male Sprague Dawley rats (312 ± 19.7 g, n = 4) were used. A CMA/12 guide cannula was stereotaxically implanted in striatum in anesthetized animals under sterile conditions according to the procedure previously reported (6). The intracerebral guide cannulae was anchored to screws (0–80 × 1/8) and skull on position with dental cement. The animal was allowed to recovery at least three days before the microdialysis experiment was performed. One day before the microdialysis experiment, the jugular and femoral veins were cannulated for drug administration and blood sampling.

Experimental Design

Study 1. Microdialysis Study of the Plasma/Brain ECF Distribution of GBP

1a. Comparison of Microdialysis Recovery and Retrodialysis Loss of [^{14}C]GBP under in Vitro *Conditions.*

Five probes were used to determine microdialysis recovery and retrodialysis loss. To estimate microdialysis recovery, a probe was placed in a beaker containing [^{14}C]GBP (2.2×10^4 dpm/mL, 0.4 μg/mL), and perfused with blank CSF solution at a flow rate of 2 μL/min using an infusion pump (Model 55-4152, Harvard Apparatus, South Natick, MA). The relative recovery was calculated by the activity in 50-μL of dialysate collected in four consecutive 30-min intervals divided by that

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pipetted out from the beaker. Retrodialysis loss of [^{14}C]GBP was measured in the same probes as used for microdialysis. In contrast to microdialysis, retrodialysis was performed where the probe was placed in blank CSF solution, but perfused with a [^{14}C]GBP solution (2.2 × 10^4 dpm/mL). Retrodialysis loss was calculated as follows (2):

$$\text{Loss} = 1 - \text{Activity in Dialysate/Activity in Perfusate} \quad (1)$$

1b. Microdialysis-Experiment in Vivo.

Rats were restrained in a finger-type cage (Centrap Cage, Fisher, Fairlawn, NJ) during the 10-hour experimental period. A microdialysis probe was inserted into the guide cannula while the probe was perfused with a [^{14}C]GBP solution (0.4 μg/mL) at a flow rate of 2 μL/min. Dialysates were collected into 300-μL capped vials every 30 min for 2 hours to estimate the retrodialysis loss *in vivo*. The probe was perfused with blank CSF and dialysate collected for another two and a half hours to wash out [^{14}C]GBP delivered into the brain and to ensure [^{14}C]GBP was entirely removed from the probe. To prevent bubbles from entering the probe, a multifunctional 10-port valve (Valco Instruments, Co. Inc., Houston, TX) was used to switch perfusate solutions.

Following the washout period, the 2 mg/mL [^{14}C]GBP solution was infused intravenously at a rate of 26.1 mL/hr for 2 min followed by a rate of 0.5 mL/hr for 6 hours. The first infusion served as a loading dose to reduce the time to reach steady state. Two minutes following initiation of drug administration (allowing dead volume in the outlet tubing to be cleared), dialysates were collected into 300-μL capped vials every 30 min until the end of infusion. Blood (200 μL) was collected into heparinized syringes from the jugular vein, before administration and at 2, 7, 15, 45, 75, 135, 195, 255, 315, and 345 minutes, and plasma harvested.

At the end of infusion, the rat was anesthetized (intravenous injection of 1 mL sodium pentobarbital, 65 mg/mL) and sacrificed by decapitation. The brain was removed immediately, cleaned of surface blood vessels and dissected on moist filter paper. Four regions of the brain, cortex and striatum on the left and right hemispheres (about 50 mg each), were obtained for analysis.

Study 2. Tissue Homogenate Study of the Plasma/Brain Tissue Distribution of GBP

Thirty six rats were administered [^{14}C]GBP (15 mg/kg) as an intravenous bolus dose via a tail vein. Six animals were sacrificed by exsanguination at each time point 30, 60, 120, 240, 360, and 480 min post dose. Blood and brain tissue (about 0.2 g) were harvested immediately and were treated as in Study 1.

Sample Analysis

Radioactivity in aliquots of dialysate (50 μL) and plasma (20 μL) were determined by liquid scintillation (Packard Series 4000, Packard Instruments, Downers Grove, IL). Brain tissue samples were digested with 2.0 ml of 0.5 N ammonium hydroxide (TS-2, Research Products International, Mt. Prospect, IL), and bleached with 0.45 mL of 30% hydrogen peroxide prior to counting. Radioactivity was reported in dpm using external standardization for quench correction, with subtraction of background counts in blank plasma, dialysate, and brain tissue samples. GBP concentration was calculated based on known specific activity. The concentration of [^{14}C] radioactivity was considered equivalent to intact GBP in brain and plasma, and equivalent to unbound GBP in plasma.(7) The concentration of GBP in brain ECF was calculated from the dialysate concentration divided by the *in vivo* retrodialysis loss obtained prior to drug administration.

Pharmacokinetic Analysis

1. BBB Permeability Estimation Using the Microdialysis-Pharmacokinetic Approach (Model 1)

Plasma concentrations of GBP were described by an open two-compartment body model with elimination from the central compartment. Transport across the BBB was described in Figure 1 as clearance (mL/min·g-brain) or permeability·area (PeA) product.(4) For this membrane-limited model it was assumed that blood flow to the brain was much greater than transport of GBP across the BBB. The brain was considered to consist of brain ECF and intracellular fluid (ICF). The four clearance terms (CL_1, CL_2, CL_3, and CL_4) denote the bidirectional clearances between plasma and brain and across intra- and extracellular membranes (IEM) in brain tissue. Two more assumptions were made for modeling: 1) transport across brain IEM was much greater than transport across the BBB, i.e. CL_3 and CL_4 were much greater than CL_1 and CL_2, and 2) tissue binding was an instantaneous process. The apparent volume of distribution in the brain with the ECF concentration as reference ($V_{e,app}$) was defined as:

$$V_{e,app} = (A_{br,tot} - V_{bl} \cdot C_{bl})/C_e \quad (2)$$

where $A_{br,tot}$ represents the total mass of drug per g-brain and V_{bl} is the volume of blood in one gram of brain, and C_e and C_{bl} are the drug concentrations in ECF and blood, respectively. Based upon the model assumptions, the number of parameters in Model 1 can be reduced from four to two, i.e., CL_1 and CL_2. The general differential mass balance equation in the ECF compartment will be:

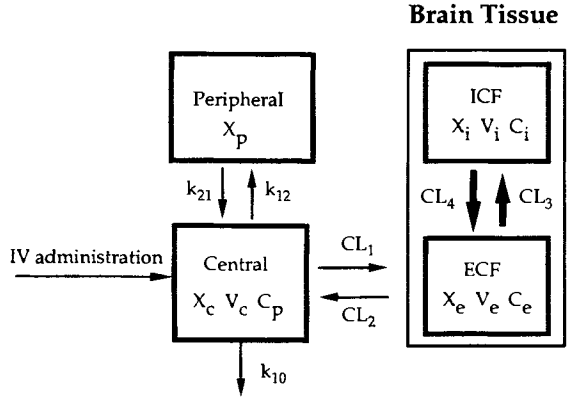

Fig. 1. Model 1: A hybrid pharmacokinetic model of drug exchange across the blood-brain barrier (BBB) and between brain extracellular fluid (ECF) and intracellular fluid (ICF) following intravenous administration.

$$V_{e,app} \cdot dC_e/dt = CL_1 \cdot C_p - CL_2 \cdot C_e \quad (3)$$

At steady-state:

$$CL_1/CL_2 = C_{e,ss}/C_{p,ss} \quad (4)$$

The parameters of Model 1 were characterized in stages by nonlinear regression using ADAPT II with Akaike Information Criteria for model selection. In the first stage, plasma concentrations were used to describe the parameters (k_{12}, k_{21}, k_{10}, and V_c) of the two-compartment open model. In the second stage, the plasma profile (C_p) defined by these parameters was used as a forcing function to generate the ECF concentrations obtained during intravenous infusion. Data was weighted using the inverse of the variance, assuming a linear relationship between concentration and standard deviation. $V_{e,app}$ was fixed in the regression analysis, and calculated from Equation 2. A value for V_{bl} was derived from the literature, that is, approximately 3% of brain tissue weight is blood, therefore, a 2-g brain in a 300-g rat contains approximately 60 μL of blood (8). The total amount of GBP in brain ($A_{br,tot}$) was obtained at the end of the experiment, and C_e determined from the last dialysate sample collected. The value for C_{bl} was calculated from the last GBP plasma sample collected assuming a blood/plasma partition ratio of 0.87 (7).

2. BBB Permeability Estimation Using the Tissue Homogenate-Pharmacokinetic Approach (Model 2)

The model for plasma to brain tissue distribution of GBP is described in Figure 2. The equation for the rate of GBP uptake into brain homogenate was previously (4) described as:

$$dC_b/dt = K_{in} \cdot C_p - (K_{out}/V_b) \cdot C_b \quad (5)$$

where C_b (μg/g-brain) is the total concentration of GBP in the brain after correction for drug concentration in the vascular blood at the corresponding time point, V_b is the physical volume of distribution of GBP in brain (assumed as 1 mL/g-brain), C_p is the plasma concentration of GBP (μg/mL), K_{in} is the clearance for influx (mL/min·g-brain) normalized to brain weight, and K_{out} is the clearance for efflux (mL/min·g-brain). Similar to Model 1, equation 5 is valid under the assumption that blood flow to the brain is much greater than transport across the BBB (K_{in}). Assuming that GBP plasma concentration-time data conforms to a two-compartment model, K_{in} and K_{out} were estimated using regression analysis as described above.

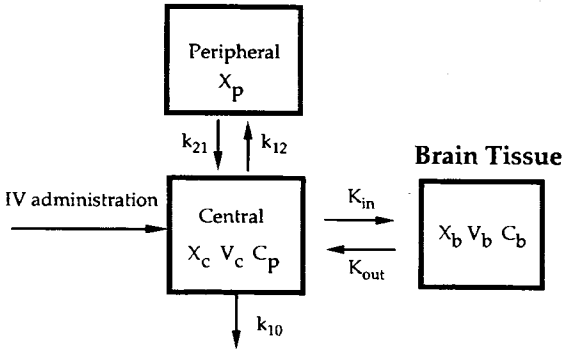

Fig. 2. Model 2: A hybrid pharmacokinetic model of drug exchange between plasma and brain based on the approach described by Takasato, *et al.* (4).

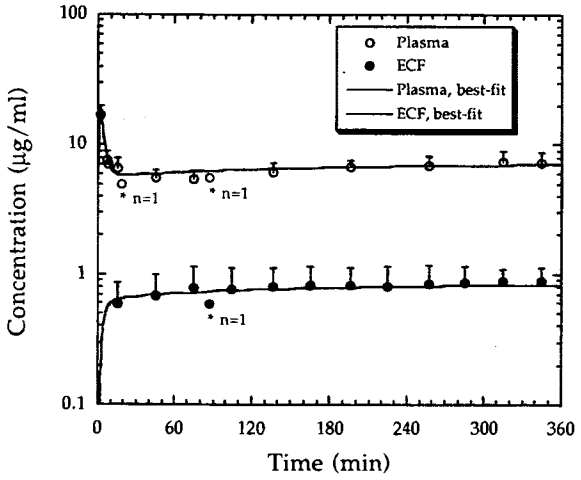

Fig. 3. Concentration (mean ± SD)-time profiles of gabapentin during intravenous infusion (52.2 mg/hr for 2 min followed by 1 mg/hr for 6 hours, n = 4).

RESULTS

The percent mean (SD) *in vitro* recovery and loss (n = 5) of GBP were 22.0 (3.4)% and 20.6 (3.4)%, respectively, and statistically not significantly different ($p > 0.05$, paired Student t-test). The *in vivo* retrodialysis loss for GBP was 8.3 (1.9)% (n = 4). No detectable amount of [^{14}C]GBP was observed after the first dialysate fraction. The minimal activity in the first post-dialysate sample (10% of C_{in}) was due to residual standard solution (0.4 μg/mL) remaining in the dialysis tubing.

The concentration-time profiles of GBP in plasma and brain ECF during infusion are presented in Figure 3. The ECF concentrations are represented graphically at the midpoint of the 30-min collection period. No significant difference in brain concentration or in brain to plasma concentration ratios was found among the four regions, right and left cortex and striatum. Therefore, brain tissue concentrations were pooled for pharmacokinetic analysis. All brain to plasma concentration ratios were significantly greater than the ECF to plasma ratios (paired Student t-test, $p < 0.05$). The mean (SD) steady-state plasma, ECF and brain tissue concentrations were 6.94 (1.15), 0.87 (0.26), and 4.45 (0.74) μg/mL, respectively. The value of $V_{e,app}$ calculated based on Equation 4 was 5.5 (2.0) mL/g-brain. Mean (SD) clearance values estimated using Model 1 are listed in Table I.

Table I. Pharmacokinetic Parameters Obtained from Brain ECF Concentration Versus Time Data During Intravenous Infusion of [^{14}C]Gabapentin to Rats (n = 4, Study 1)

			Relative SE Estimate (%)	
Parameter	Mean	SD	Mean	SD
CL_1 (mL/min·g-brain)	0.044	0.021	25.4	11.5
CL_2 (mL/min·g-brain)	0.376	0.196	27.3	11.5
$V_{e,app}$ (mL/g-brain)*	5.5	2.0		

* Calculated using Equation 4.

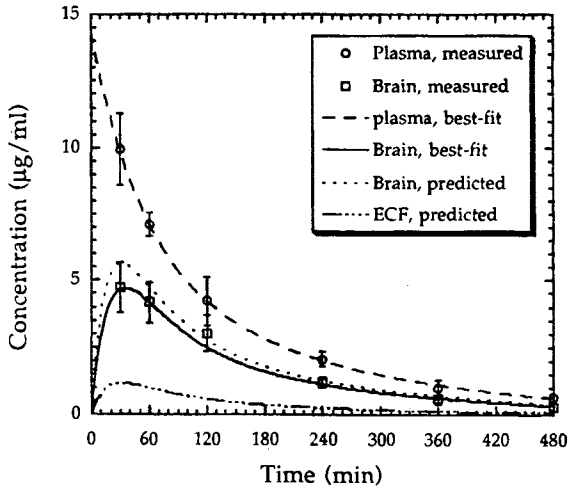

Fig. 4. Gabapentin concentration (mean ± SD)-time profiles (measured and best fit) after administration of a 15 mg/kg intravenous bolus dose. The predicted ECF concentrations were simulated using the best-fit plasma concentration as a driving function and based on parameters (CL1 and CL2) obtained in Study 1. The predicted brain concentration was calculated by dividing the predicted ECF concentrations by a common factor of 0.206 obtained in Study 1.

The concentration-time profiles of GBP in plasma and brain tissue after intravenous bolus injection are plotted in Figure 4. Based upon Model 2, K_{in} and K_{out} were estimated (Table II). Assuming that drug distribution occurred instantaneously in the brain as assumed in Model 1, ECF concentrations were simulated using the plasma concentration as a driving function. Thereafter, the brain concentrations were calculated by dividing the simulated ECF concentrations by a common factor of 0.206 (ECF to brain concentration ratio, 0.206 (0.091) obtained in Study 1). As plotted in Figure 4, the calculated brain concentrations are very close to those measured in brain tissue, demonstrating that CL_3 and CL_4 must be much greater than CL_1 and CL_2 as assumed in Model 1. Back calculated ECF concentrations were determined by multiplying measured brain concentrations by a factor of 0.206, and the influx and efflux permeabilities (CL_1 and CL_2) were estimated using Model 1 (Table II).

Table II. Pharmacokinetic Parameters Obtained from Brain Homogenate Concentration Versus Time Data During Intravenous Bolus Administration of [^{14}C]Gabapentin to Rats (n = 36, Study 2)

Model 2	Mean	Relative SE Estimate (%)
K_{in} (mL/min·g-brain)	.028	50
K_{out} (mL/min·g-brain)	.056	49
Model 1*		
CL_1 (mL/min·g-brain)	.035	63
CL_2 (mL/min·g-brain)	.351	61

* Parameters were calculated using Model 1. GBP ECF concentrations were calculated by multiplying the measured brain concentrations by a factor of 0.206, the ECF to brain concentration ratio obtained in Study 1. The value for $V_{e,app}$ was fixed and taken from Study 1 also (5.5 mL/g-brain).

DISCUSSION

Microdialysis was used to monitor free GBP concentrations in the brain ECF of conscious rats. This method allowed for the study of GBP central nervous system (CNS) pharmacokinetics in individual animals. The *in vivo* microdialysis probe recovery for GBP was determined by the retrodialysis method (2,9), referred to by others as the internal standard or internal reference method (10,11). In this method, the relative loss of compound from the perfusate is assumed to be equal to microdialysis recovery. This assumption was found to be valid for GBP under *in vitro* conditions. The relative loss of GBP *in vivo* was approximately one half to one third of the *in vitro* recovery, and is likely due to a difference in diffusivities for GBP in water versus tissue. *In vivo* recovery estimated by retrodialysis loss is stable for up to 24 hours and was therefore considered reliable over the 10-hour experimental period (9). Microdialysis with recovery calibrated by retrodialysis therefore provided quantitative information about GBP concentrations in brain ECF.

The steady-state ECF to plasma concentration ratio (13.4 ± 6.9%) was similar to the CSF to plasma concentration ratio (10–20%) in patients at steady-state (12). Based on the pharmacokinetic analysis (Model 1), the predicted brain tissue concentrations agreed well with the concentration-time course of drug measured in brain tissue (Figure 4). Therefore, the assumption that clearance across IEM was much greater than clearance across the BBB appears valid, and is consistent with CNS physiology. The surface area (A) of the BBB, approximately 100 cm^2/g (13), is considerably less than the estimated surface area for IEM, 1.9×10^4 cm^2/g, assuming the average cell size in brain is within the same range as for red blood cells (14,15). Further, the distance between plasma and brain ECF (total of 4–6 lipid bilayers from at least four different cell types, endothelial cell, pericyte, astrocyte foot and neuron, and one layer of basement membrane (16)) is much greater than across the single lipid bilayer for IEM. Therefore, the product of Pe (diffusivity/distance) and A across the BBB would be much smaller than that across the brain IEM for a compound which passes through cell membranes passively. Verification of this rational is also observed with the difference in clearance of GBP into glial cells (20 mL/min·g) *in vitro* (17), with clearance between plasma and brain *in vivo* (CL_1 and CL_2, 0.044 and 0.376 mL/min·g, respectively).

The estimated clearance values between plasma and brain may be considered as a composite of passive diffusion and carrier-mediated transport across the BBB, and diffusion and/or bulk flow between ECF and CSF. However, since ECF bulk flow (0.2–0.3 μL/min·g-brain), CSF turnover rate (2–3 μL/min·g-brain), and diffusion within the brain are normally very small (8,18), the estimated values, CL_1 and CL_2, essentially represent the clearance across the BBB directly. The value for CL_1 is similar to the lower range of permeability values reported for the large neutral amino acid (LNAA) leucine (0.047 to 0.301 mL/min·g), a system L substrate (19). Although GBP was designed as an analogue of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA) (20), in a pharmacokinetic sense, GBP behaves like a LNAA. GBP is transported by system L with Km values similar to those for LNAAs across the gut wall (21), and into astrocytes and cerebellar granule cells (17). It has also been proposed that GBP is transported across the blood-

brain barrier by system L (6). Because the clinically relevant plasma concentrations of GBP (approximately 10–100 μM) are in the typical range for LNAAs (22), it is possible that the apparent influx permeability of GBP across the BBB may decrease during periods of hyper-aminoacidemia, for instance following a high protein meal, or increase during periods of hypo-aminoacidemia caused by carbohydrate-induced hyperinsulinemia. This potential nutrient-drug interaction is similar to that described for the effect of diet on L-DOPA brain permeability (23).

Because the concentration of GBP in brain homogenate was much higher than in brain-ECF, the brain homogenate method underestimated GBP efflux permeability. For the same data generated in study 2 but calculated differently (Model 1 versus Model 2, Table II), K_{in} was nearly identical to CL_1, however, K_{out} was much smaller than CL_2. Therefore, knowledge of intracellular accumulation is significant for interpretation of data generated from brain homogenate alone with respect to efflux permeability. Further, the standard errors of all estimated parameters (Table II), where 36 animals were used, were much higher than that determined in Study 1 where only four animals were used (Table I). The efflux clearance of GBP was about 8-fold greater than the influx permeability, suggesting that transport of GBP across the BBB was asymmetric. System L is believed to be symmetrically distributed across luminal and abluminal membranes of brain capillaries (24). GBP efflux from brain may therefore be mediated in part by an entirely different transport system. System A exists primarily on abluminal membranes, and phenylalanine and leucine have been observed to inhibit system A transport suggesting some overlap of the two systems (24). However, the interaction of GBP in other transport systems has yet to be defined.b

The rapid uptake and accumulation of GBP into brain parenchyma (17) and tissue binding sites (25) relative to BBB permeability resulted in a large GBP volume of distribution in brain, $V_{e,app}$, 5.5 mL/g-brain. If a compound is unbound to tissue and has the same concentration in ICF and ECF, then $V_{e,app}$ will be equal to the sum of ICF and ECF, i.e. about 0.8 mL/g-brain assuming 20% of brain is protein (4). This is the case for zidovudine (3). $V_{e,app}$ was critical for modeling the data generated from microdialysis because it decreased the number of parameters in the model from four to two. Based upon Eq. 4, the variability in $V_{e,app}$ may be affected by the estimated blood volume in the brain. For GBP, since the total brain to blood concentration ratio (0.75) was very high, the variability in $V_{e,app}$ introduced by error in estimating the V_{bl} value from the literature (3% of brain) should be negligible. However, for compounds with poor BBB permeabilities and therefore having blood concentrations much higher than the real tissue concentration, different values for V_{bl}, 1–5%, (20,21) may result in large errors in calculated $V_{e,app}$ values.

In summary, this study demonstrates that the microdialysis-pharmacokinetic approach can be used to determine simultaneously the influx and efflux permeabilities of a compound across the BBB. The bidirectional permeabilities of GBP thus estimated have potential clinical importance. The assumption that transport between extra- and intracellular space is more efficient than transport across the BBB was justified using data generated by the brain tissue homogenate method. This assumption is reasonable for most compounds since transport across the BBB will commonly be the rate limiting step for distribution of drug to the brain, rather than distribution between extra-and intracellular space. The new term defined, $V_{e,app}$, facilitates a pharmacokinetic estimation of BBB influx and efflux permeabilities using data generated by microdialysis. A $V_{e,app}$ value greater than unity indicates an intracellular accumulation or tissue binding, which, if not considered, will lead to underestimation of the efflux permeability using the tissue homogenate-pharmacokinetic approach. However, it should be mentioned that the microdialysis-pharmacokinetic approach did not consider the effect of drug metabolism in the brain, which was not required for GBP. If brain is also an elimination organ, characterization of the metabolism under *in vitro* conditions may be necessary since *in vivo* metabolism and efflux can not be distinguished unless very rich data on metabolites in both plasma and ECF are obtained.

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