

Carbamazepine (CBZ) Distribution in the Central Nervous System of Freely Moving Rats: Correlation Between Brain Extracellular Fluid (ECF) and Cerebrospinal Fluid (CSF) Exposure

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ABSTRACT

To investigate whether exposure of the anticonvulsant CBZ in the brain ECF and CSF are comparable, brain ECF concentrations of CBZ were determined by quantitative intracerebral microdialysis and compared to results from CSF collection studies^[1]. A brain probe was surgically implanted into the striatum and the femoral vein and artery were catheterized for drug administration/blood replacement and blood sampling, respectively. The probe was perfused with artificial CSF (0.5 $\mu\text{L}/\text{min}$) and dialysate samples collected every 20 min. Plasma was sampled at the midpoint of the dialysate collection interval. CBZ and its active metabolite, 10,11-epoxide CBZ (ECBZ), in plasma and dialysate samples were quantified by LC-MS/MS analysis. The unbound plasma and brain ECF concentrations of CBZ and ECBZ, were determined both after a single *i.v.* bolus dose of 4 mg/kg CBZ and at steady-state during constant rate *i.v.* infusion of 1 mg/h/kg CBZ. Since *in vitro*, the relative loss (RL) of CBZ, ECBZ and deuterated CBZ (d10-CBZ) from the probes equalled the relative recovery (RR), and RL of d10-CBZ equalled the RR of CBZ and ECBZ, the RL of d10-CBZ was used to correct for recovery *in vivo*. The RL of CBZ, ECBZ and d10-CBZ *in vivo* were also determined following a 24 hour wash-out period. *In vivo*, the RL of d10-CBZ equalled the RL of CBZ, however, the RL of ECBZ was only half of that of brain ECF and unbound plasma were 0.76 \pm 0.13 and 0.84 \pm 0.11, respectively (n=7). These values are consistent with the ratios of the brain ECF to unbound plasma concentrations determined at steady-state (0.60 \pm 0.15 for CBZ and 0.64 \pm 0.12 for ECBZ, n=7). The CSF to unbound plasma AUC ratio for CBZ determined previously (0.71 \pm 0.08 (n=7))^[1], compares favourably to these values, indicating that CSF exposure is an appropriate surrogate for brain ECF exposure of CBZ.

INTRODUCTION

The brain distribution of a CNS drug and its metabolites is impeded by the presence of blood-brain barrier (BBB) and blood-cerebrospinal fluid (BCSFB) barriers, two barriers equipped with tight junctions and a panel of efflux transporters. Hence, in relation to the plasma counterparts, the biophase drug and metabolite concentrations in the brain are much better related to therapeutic and toxic effects. However, the acquisition of such data requires microdialysis, a technique that is highly sophisticated and resource-demanding. The concentration in cerebrospinal fluid (CSF) is often used as a "surrogate" of that in brain ECF, since no tight junction exists for the ependymal cells lining the brain ECF and CSF. A terminal or serial CSF collection^[1] are more convenient, economical, and straightforward and amenable to early drug discovery paradigm. Nevertheless, it remains debatable whether CSF concentrations are always good surrogates for brain concentrations^[2], in view of the different pattern of transporter expression in BBB and BCSFB and the complexity of CSF formation, trafficking, and turnover. In this study, the CNS distribution of carbamazepine and its active metabolite, 10,11-epoxide CBZ, were investigated under a transient condition and steady state. These results were compared to those obtained by a serial CSF collection^[1].

MATERIALS AND METHODS

***In vitro* feasibility of microdialysis for carbamazepine.** The relative loss (RL) and recovery (RR) of CBZ, ECBZ, and deuterated CBZ (d10-CBZ) were determined *in vitro*.

Surgery. At least two days before the study, a microdialysis guide cannulae (CMA-12) was implanted into striatum region of rat brain (anteroposterior +0.2 mm, lateral -2.8 mm, ventral -3.6 mm; all relative to bregma) and the femoral vein and artery were catheterized.

Microdialysis. Twelve hours before the study, a microdialysis probe (CMA12, 4 mm) was inserted into brain striatum and the rat was tethered in a CMA-120 free moving system. On day 1, the retrodialysis of d10-CBZ (1 $\mu\text{g}/\text{mL}$ in aCSF) was started 2 hr before the dosing. The rat was given an *i.v.* bolus of CBZ (4 mg/kg) only or an *i.v.* bolus (1 mg/kg) followed by a constant *i.v.* infusion (1 mg/h/kg). For *i.v.* bolus administration, the blood was sampled at pre-dose, 5, 10, 20, 30, 50, 70, 90, 110, 130, 190, 250, 310, 370, and 490 min post-dosing. For *i.v.* infusion, the steady state was reached at 6 hr and the blood was sampled at 6, 7, 8, 9, and 10 hr. Dialysate was collected every 20 min. Upon completion of sample collection, the perfusate was switched to aCSF and an overnight washout was allowed to deplete remaining CBZ, ECBZ, and d10-CBZ. On day 2, the perfusate was switched to a mixture of CBZ, ECBZ, and d10-CBZ (1 $\mu\text{g}/\text{mL}$ in aCSF) and the dialysate was collected every 20 min from 4 to 8 hrs. All the samples were assayed by LC-MS/MS.

Data Analysis. The relative recovery and loss were calculated according to Eq. 1 and 2, respectively, as shown,

$$RR = \frac{C_{\text{dialysate}}}{C_{\text{u,brain ECF}}} \quad (\text{Eq. 1}) \quad RL = 1 - \frac{C_{\text{dialysate}}}{C_{\text{perfusate}}} \quad (\text{Eq. 2})$$

where $C_{\text{dialysate}}$ and $C_{\text{perfusate}}$ are the concentrations in dialysate and perfusate, respectively, and $C_{\text{u,brain ECF}}$ is the unbound concentration in brain ECF (*in vivo*) or in the solution that surrounds the probe (*in vitro*). The unbound concentration in plasma ($C_{\text{u,plasma}}$) was obtained by multiplying the unbound fraction ($f_{\text{u,p}}$, 0.33 for CBZ, in house data; 0.70 for ECBZ, literature value^[3]) to the total concentration in plasma. $C_{\text{u,brain ECF}}$ was obtained by dividing $C_{\text{dialysate}}$ with real-time RR that was monitored with retrodialysis of d10-CBZ. $C_{\text{u,plasma}}$ and $C_{\text{u,brain ECF}}$ were analyzed non-compartmentally with WinNonlin Pro 4.0 (Pharsight Corp., Mountainview, CA).

RESULTS

1. The feasibility of microdialysis for assessing the CNS distribution of CBZ and ECBZ were verified both *in vitro* and *in vivo* (Table 1). *In vitro*, RRs of CBZ, ECBZ, d10-CBZ equalled their respective RLs. RRs and RLs for all three compounds did not differ significantly and the RL of d10-CBZ equalled the RR of CBZ. *In vivo*, RL of d10-CBZ equalled that of CBZ and this validated d10-CBZ as a good internal standard in retrodialysis for the measurement of RR of CBZ. RL of ECBZ was about 45% of RLs of CBZ and d10-CBZ.

2. Following an *i.v.* bolus dosing, CBZ rapidly appeared in the brain ECF and peaked at ~30 min (Figure 1). The post-peak $C_{\text{u,brain ECF}}$ of CBZ approximated the unbound concentration of CBZ in plasma ($C_{\text{u,plasma}}$). In contrast, the metabolite ECBZ appeared in plasma, peaked at about 2 hr, and then slowly decayed. ECBZ also appeared in the brain ECF rapidly but at a much lower concentration. ECBZ in the brain ECF peaked and decayed in parallel to that in plasma. In the terminal portion, $C_{\text{u,brain ECF}}$ of ECBZ was almost identical to $C_{\text{u,plasma}}$ of ECBZ.

3. The AUC ratios of unbound CBZ and ECBZ (Table 2) in brain to plasma following an *i.v.* bolus were consistent with the unbound concentration ratios at the steady state. More importantly, both ratios matched the AUC ratio of CSF concentration to unbound plasma concentration^[1]. All the ratios were slightly less than unity, suggesting that the involvement of efflux transporters in the CNS penetration of both CBZ and ECBZ, if exists, should be limited.

Table 1. *In vitro* and *in vivo* probe calibration. Data represent the mean \pm S.D. relative recovery (RR) and relative loss (RL) for each solute.

Solutes	<i>In vitro</i> @ 1.25 $\mu\text{L}/\text{min}$ (n = 5)		<i>In vivo</i> @ 0.5 $\mu\text{L}/\text{min}$ (n = 11)
	RR	RL	RL
CBZ	0.53 \pm 0.06	0.56 \pm 0.03	0.56 \pm 0.08
ECBZ	0.53 \pm 0.05	0.52 \pm 0.04	0.25 \pm 0.05
d10-CBZ	0.56 \pm 0.04	0.53 \pm 0.04	0.55 \pm 0.07

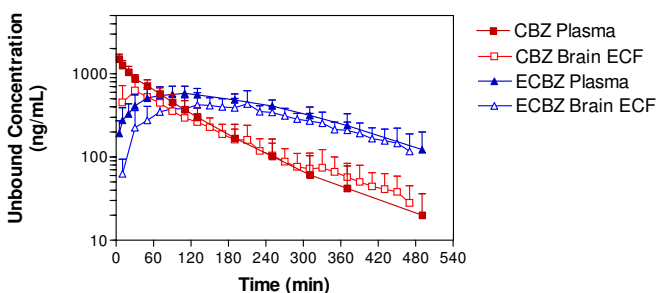


Figure 1. Time dependent profiles of unbound concentration (mean \pm S.D.) of CBZ and ECBZ in plasma and brain ECF following an *i.v.* bolus (4 mg/kg) dosing to Sprague-Dawley rats (n = 7)

Table 2. Ratios of area under the unbound concentration-time curve (0 to infinity) of CBZ and ECBZ in plasma and brain ECF under transient condition and the unbound concentration ratio in brain and plasma at the steady state. Data were presented as mean \pm S.D..

Ratios	CBZ	ECBZ
$AUC_{\text{u,brain ECF}}/AUC_{\text{u,plasma}}$	0.76 \pm 0.13	0.84 \pm 0.11
$C_{\text{u,brain,ss}}/C_{\text{u,plasma,ss}}$	0.60 \pm 0.15	0.64 \pm 0.12
$AUC_{\text{CSF}}/AUC_{\text{u,plasma}}$	0.71 \pm 0.08	-

CONCLUSIONS

Both the AUC ratio of unbound CBZ by microdialysis under transient condition and the unbound concentration ratio at the steady state in brain ECF to that in plasma were consistent with the AUC ratio of CSF concentration to unbound plasma concentration^[1]. This suggests that CSF concentration is a good surrogate for brain ECF concentration for CBZ, a compound that displays good CNS penetration and limited carrier-mediation.

REFERENCES

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FOOTNOTES

- ¹ These two authors made equal contribution to this work.