

Quantitative Microdialysis for the Study of Drug Distribution in the CNS of Freely Moving Rats

Background

The determination of drug distribution into the brain in drug discovery and development is of vital importance. Sufficient brain distribution for Central Nervous System (CNS)-targeted drugs is required for exhibiting desirable pharmacological effects, whereas brain distribution of non-CNS-targeted drugs should be minimized to avoid possible CNS side effects and toxicity. The blood-brain and the blood-cerebrospinal fluid (CSF) barriers limit drug CNS entry due to the presence of tight junctions, drug metabolizing enzymes, and drug efflux transporters. The presence of these barriers in conjunction with the physicochemical properties of a drug determines the rate and extent of its CNS distribution. Several methods, such as brain homogenization, CSF collection and microdialysis, have been developed to study drug CNS distribution. In contrast to the other methods, quantitative intracerebral microdialysis is a direct method for determining the unbound drug concentration in the extracellular fluid (ECF) of different brain regions over time. In addition, biomarkers such as dopamine and serotonin may be measured simultaneously and a good pharmacokinetics-pharmacodynamics (PK-PD) relationship can be built using this method.

Quantitative Intracerebral Microdialysis in Freely Moving Rats

To perform microdialysis, a small probe is implanted into a specific region of the brain. The lower portion of the probe is composed of a semi-permeable membrane that allows the unbound drug molecules to diffuse from the ECF into the probe and vice versa. The probe is perfused at a constant rate with artificial CSF (aCSF). Unbound drug molecules in the ECF diffuse across the probe membrane and appear in the dialysate. As this is not a static system, the concentration of drug in the dialysate ($C_{\text{dialysate}}$) is a fraction of that unbound in the ECF ($C_{\text{u, ECF}}$). This fraction, referred to as the relative recovery (RR), must be determined in order to estimate $C_{\text{u, ECF}}$.

In delivery (retrodialysis) mode, aCSF containing the drug is delivered through the probe. A fraction of the concentration of drug delivered ($C_{\text{perfusate}}$) diffuses into the brain ECF, with the remainder recovered in the dialysate. The fraction lost into the ECF is referred to as the relative loss (RL) and equals $1 - C_{\text{dialysate}}/C_{\text{perfusate}}$. Due to the symmetrical nature of the diffusion, RR should be equal to RL unless non-specific binding of the drug to the apparatus tubing and/or probe occurs. If RL proves to be equal to RR *in vitro*, RL *in vivo* (measured either before or after the PK study when no test compound is present in the body) can be used to estimate RR and to correct the dialysate data. Hence, it is necessary to ascertain whether RL equals RR *in vitro* prior to an *in vivo* study.¹ Alternatively, the

radiolabeled or deuterated form of the test compound or a structural analogue (a calibrator), may be used to obtain a real-time estimate of RR during a PK study, given that the RL of the calibrator equals the RR of the test compound.

NoAb Microdialysis Service

NoAb BioDiscoveries has established a quantitative intracerebral microdialysis system in freely moving rats. In this system, a brain probe is surgically implanted into a specified region of the brain and the femoral vein and artery are catheterized for drug administration, blood sampling and blood replacement. Artificial CSF is perfused by a syringe pump into the probe at a designated flow rate (range 0.5-2 $\mu\text{L}/\text{min}$) and the dialysate sample is collected every 20 minutes. Plasma is sampled at the midpoint of the dialysate collection interval. Analytes in the plasma and dialysate samples are quantified by LC-MS/MS analysis. Prior to the *in vivo* study, an *in vitro* feasibility study is conducted to prove that RL equals RR for each test compound. When a calibrator is used, it is also necessary to prove that RL of the calibrator equals the RR of the test compound.

Experimental Results

As an example, the unbound plasma and brain striatum ECF concentrations of carbamazepine (CBZ) and its active metabolite, 10,11-epoxide carbamazepine (ECBZ), were determined both after a single *i.v.* bolus dose of 4 mg/kg CBZ and at steady-state during *i.v.* infusion of 1 mg/kg/h CBZ to rats. Deuterated CBZ (d10-CBZ) was used as a calibrator throughout the study. The RL of CBZ, ECBZ and d10-CBZ were also determined following a 24 hour wash-out period. As shown in Table 1, at a perfusate flow rate of 1.25 $\mu\text{L}/\text{min}$ the *in vitro* RL equaled the RR for all three compounds and the RL of d10-CBZ equaled the RR of CBZ and ECBZ. *In vivo* the RL of d10-CBZ equaled the RL of CBZ; however, the RL of ECBZ is only half of that of d10-CBZ and CBZ. Following the bolus dose of CBZ, both CBZ and the formed metabolite ECBZ, exhibited good brain distribution (Figure 1). The ratios of the area under the unbound concentration *versus* time curve (AUC) for brain ECF and plasma are 0.76 ± 0.13 and 0.84 ± 0.11 , respectively, for CBZ and ECBZ (n=7). These values are consistent with the ratios of the unbound brain ECF to unbound plasma concentrations determined at the steady-state (0.60 ± 0.15 for CBZ and 0.64 ± 0.12 for ECBZ, n=6). These ratios are also consistent with the CSF to unbound plasma AUC ratio (0.71 ± 0.07).²

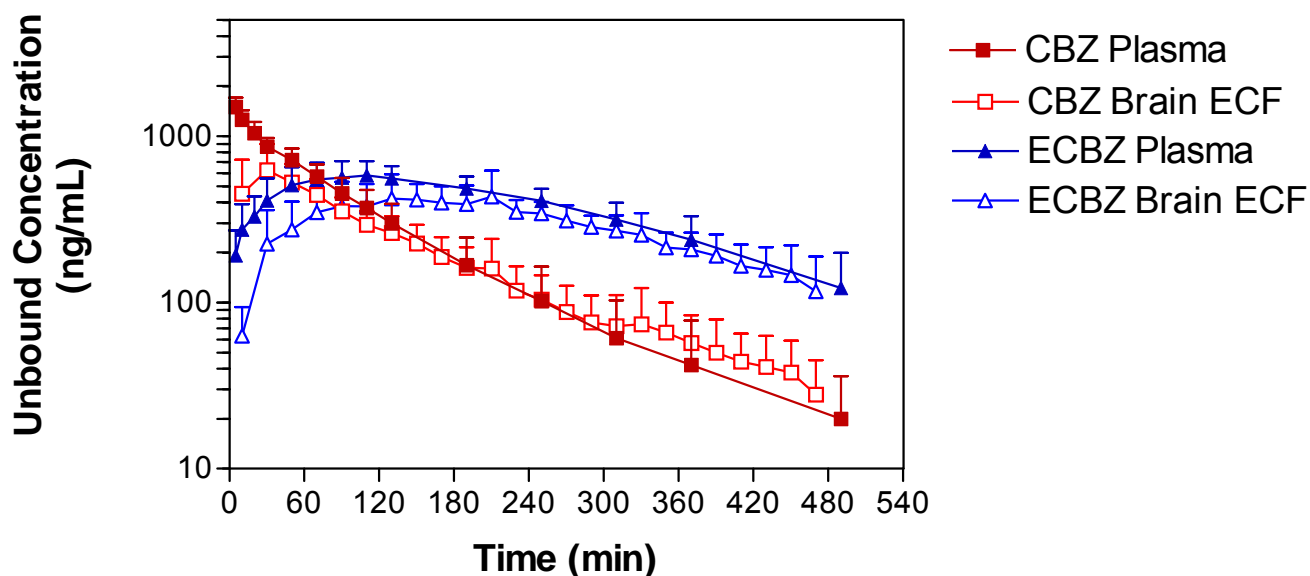
¹ Chaurasia CS, et al. (2007) AAPS-FDA Workshop White Paper: Microdialysis principles, application and regulatory perspectives. *Pharm Res* 24:1014-25: <http://www.aapsi.org/view.asp?art=aapsi0901006>

² Saldivia V, et al. (2008) Pharmacokinetics of Carbamazepine in Serial Samples of Plasma and CSF Following Intravenous Administration to Conscious Rats. *Drug Metabolism Reviews* 40(3):108-9 http://www.noabbiodiscoveries.com/html/invivo/invivo_articles.html

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. Perfusate flow rates through the probe were 1.25 μ L/min for *in vitro* measurements and 0.5 μ L/min for *in vivo* measurements.

Solutes	<i>In vitro</i> @ 1.25 μ L/min (n = 5)		<i>In vivo</i> @ 0.5 μ L/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: Mean (+S.D.) unbound concentration *versus* time profiles of CBZ and ECBZ in plasma and brain ECF following *i.v.* bolus administration of 4 mg/kg CBZ to 7 rats.



Quantitative intracerebral microdialysis provides a direct measurement of drug and metabolite unbound concentrations in the extracellular space adjacent to the drug target. We are confident that this service will be a valuable addition to our other CNS distribution studies, including brain homogenate and serial CSF and plasma collection.

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