Pre-equilibrium In vivo Soild Phase Microextraction for Monitoring Drug Concentration Changes in Brain Tissue

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Abstract

An advantage of pre-equilibrium solid phase microextraction (SPME) method has been utilized to quantitatively determine the concentrations of carbamazepine from the cortex and striatum regions of the brain of freely moving rats. The method was validated with a parallel microdialysis sampling and the results compared with conventional plasma sample analysis. A 10-min extraction was employed for SPME extractions while 30 min sampling time was required for MD. A pharmacokinetic studies of carbamazepine in both steady and dynamic states was investigated using microdialysis and SPME. Comparable results were obtained for both methods. Drug concentrations in the cortex and striatum measured with solid phase extraction method did not show any significant differences. The CV% recorded for both methods were ≤ 15.

Introduction

Significant advances in brain research and knowledge of brain related disorders have improved over the years. However, the fact still remains that the world's leading cause of disability is related to neurological disorders. This may be due to the fact that the effectiveness of therapeutic drugs is often complicated by the presence of blood-brain barrier in conjunction with the drug physicochemical properties, brain tissue mass and the action of enzymes among others. The likely result is that the distribution of the drug after delivery may be significantly perturbed. Therefore knowledge of the distribution of a drug in different regions of the brain is of vital importance to drug discovery and development, i.e., reliable *in vivo* sampling methods with good spatial resolution will be an invaluable asset to drug discovery and development.

Microdialysis, a sample preparation method has been used extensively for *in vivo* brain tissue research. The method has been successful because it enables identification of chemical changes in the brain and thus facilitates the discovery of drug targets for therapeutic purposes. However, MD is often characterized with very low temporal resolution. In addition, to monitor different brain regions simultaneously, MD requires the use of multiple probes for sampling. The process not only requires multiple surgeries to the performed on a single rat but also results in very poor spatial resolution.

Solid phase microextraction (SPME) is an equilibrium extraction method based the partitioning of the analyte between the extraction phase and the sample matrix. Typically equilibrium SPME extractions often lead to low temporal resolution. However, recent advances of the method and the availability of highly sensitive detection systems, for example the mass spectrometer, have made it possible for pre-equilibrium shorter extraction times (< 1 min in some cases) with improved temporal resolution.

Objective

This demonstrates the use of pre-equilibrium 10-min SPME method with a single fiber coating (7 mm coating length) to simultaneously measure the amount of carbamazepine drug distributed within the cortex and striatum of freely moving rats. The SPME method was validated with MD by simultaneously sampling from both brain hemispheres (Fig 1)

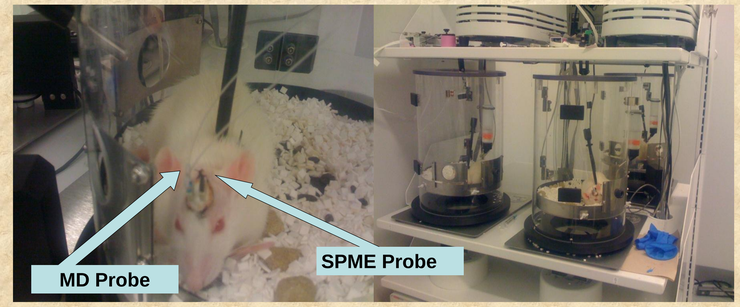
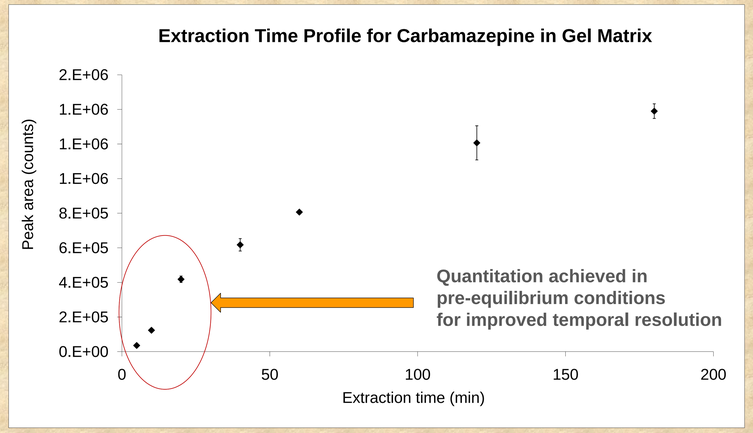


Figure 1 In vivo monitoring of carbamazepine in the brain of freely moving rats

SPME Method Development

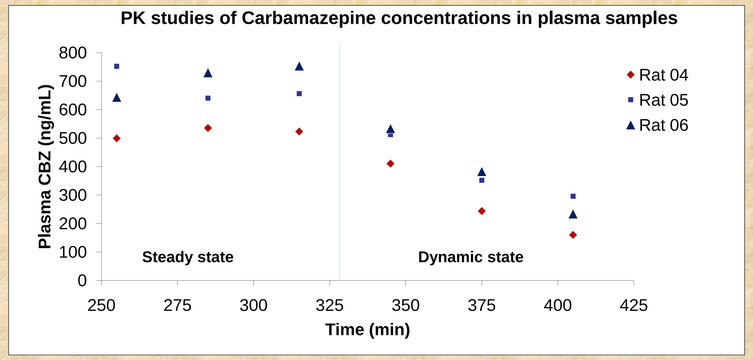


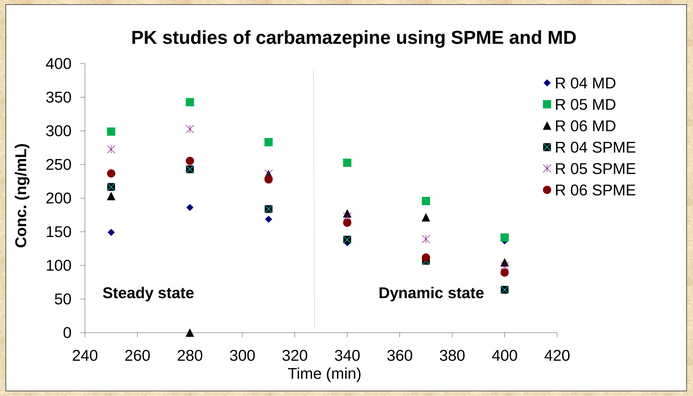
Summary of Experimental Procedure

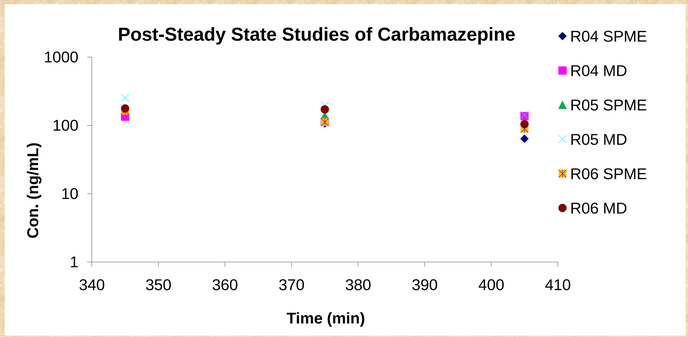
- **Simultaneous monitoring of carbamazepine from both left and right hemispheres of the rat brain was performed by using SPME (7 mm) and MD probes (2 mm)**
- **MD probes where surgically placed in the frontal cortex section only of the rat brain**
- **SPME probes were surgically located within the frontal cortex and striatum of the rat brain**
- **Sampling time: MD dialysates collected @ 30 min interval. SPME extraction time was ONLY 10 min**
- **Total drug time monitoring was 7 hr. Drug monitoring was performed in both steady (constant infusion of carbamazepine) and dynamic states.**
- **Carbamazepine dosing: 1.5 mg/kg *i.v.* bolus**
- **All samples were analyzed using HPLC coupled to a tandem mass spectrometer**

NOTE: Blood samples were also collected at 30 min intervals for plasma analysis using traditional protein precipitation method. All blood samples were collected in between the time for MD dialysate samples.

Results: Pharmacokinetic Studies







Results: Drug concentrations in cortex and striatum regions

	Rat 4		Rat 5		Rat 6	
	Cortex	Striatum	Cortex	Striatum	Cortex	Striatum
MD	-	168	-	308	-	219
STDEV	-	18	-	31	-	23
SPME	215	212	250	271	221	240
STDEV	14	30	33	33	33	14

Pre-equilibrium SPME method successfully used to measure carbamazepine concentrations simultaneously from different regions of the brain

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