Free phenytoin concentration in brain ECF

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FREE PHENYTOIN CONCENTRATION MEASUREMENT IN BRAIN EXTRACELLULAR FLUID: A PILOT STUDY.

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Abstract

We investigate the relationship between brain extracellular fluid free phenytoin concentration and plasma free phenytoin concentration in adults with acute brain injury. Daily cerebral microdialysate free phenytoin concentration was measured in eight adults with acute brain injury and compared to simultaneous measurement of plasma free phenytoin concentration.

The group data revealed no significant correlation between microdialysate and plasma free phenytoin concentration (r=0.34, p=0.41). However in two patients, with a sufficient number of samples for intra-individual analysis, there was a significant correlation between microdialysate and plasma free phenytoin concentration (r=0.92, p<0.001 and r=0.88, p<0.01). In vitro microdialysis relative recovery for phenytoin was 2.1%.

In the context of acute brain injury, measurement of free plasma phenytoin concentration may not provide an accurate reflection of regional brain extracellular fluid free phenytoin concentration and may have limitations with respect to achieving reproducible brain extracellular fluid free phenytoin concentrations. This has implications for dosing regimens relying on plasma phenytoin levels.

Introduction

Seizures are a well recognised complication of acute brain injury (ABI), and have been described in the context of both traumatic brain injury (TBI) and subarachnoid haemorrhage (SAH)[1-5]. Patients with TBI often suffer from seizures during neurocritical care and these seizures can occur despite the administration of phenytoin[6]. The 5 year cumulative incidence of seizures after severe TBI has been estimated as 10% with the highest risk period being in the first year after injury when the standardised incidence ratio is 95.0[7]. After SAH the incidence of in-hospital seizures has been reported as 7%[8]. Seizures in the acute phase after acute brain injury have potentially deleterious effects due to exacerbation of secondary brain injury and seizure management is therefore an important component of neurocritical care.

Phenytoin is a first line treatment for seizures post ABI but potential toxicity requires that drug level monitoring forms an integral part of dosing strategy. It is a drug with a high degree of protein binding and dosing is therefore traditionally based on the plasma concentration of free non-protein bound phenytoin ([$Phen_{PLASMA}$]). However, the sites of action of phenytoin as an anti-epileptic drug are at the neuronal sodium and calcium channels[9] and drug concentrations at these sites may be poorly reflected by plasma unbound drug concentration. Unbound phenytoin concentration in brain extracellular fluid ([$Phen_{ECF}$]) may therefore provide a more pertinent measure of anti-

epileptic effect because of individual differences in free phenytoin concentration gradient between the plasma and the brain extracellular fluid (ECF)[10].

Cerebral microdialysis is an established technique that allows focal measurement of brain ECF biochemistry and is a routine part of multimodality monitoring on the neurocritical care unit[11]. Microdialysis also allows estimation of drug concentrations and has previously been used to measure free phenytoin concentration in the brain ECF of two patients[10], and blood of one patient[12], with epilepsy.

The aim of this pilot study was to investigate patients treated with phenytoin and undergoing cerebral microdialysis monitoring after ABI in order to examine the relationship between [$Phen_{PLASMA}$] and cerebral microdialysate free phenytoin concentration ([$Phen_{MD}$]).

Materials and Methods

In Vitro Recovery Study

A solution of approximately 7.5 µmol/l phenytoin was prepared by diluting phenytoin sodium in compound sodium lactate solution (sodium 131 µmol/l, potassium 5 µmol/l, calcium 2 µmol/l, chloride 111 µmol/l, lactate 29 µmol/l, Baxter Healthcare Ltd, UK). 2 microdialysis catheters (CMA 70, CMA/Microdialysis, Solna, Sweden) with 10 mm dialysis membrane length and 20 kDa molecular weight cut-off were placed in the phenytoin solution at the same level to avoid any hydrostatic gradients. The catheters were

perfused with artificial CSF (NaCl 147 mmol/l, KCl 2.7 mmol/l, $CaCl_2$ 1.2 mmol/l $MgCl_2$ 0.85 mmol/l, Perfusion Fluid CNS, CMA/Microdialysis), using a CMA 106 pump (CMA/Microdialysis), at a rate of 0.3 µL/min. Phenytoin concentration was measured in the phenytoin solution pre and post the recovery study, and in the microdialysate, using the assay described below. Relative recovery for each catheter was calculated by expressing the microdialysate phenytoin concentrations as a percentage of the mean of the pre and post study phenytoin concentrations in the solutions.

In Vivo Patient Study

This study was approved by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology and, as all subjects were unconscious at the time of the study, written assent was obtained from their personal representatives. The inclusion criteria were a diagnosis of either traumatic brain injury or subarachnoid haemorrhage, the presence of cerebral microdialysis monitoring and pharmacological treatment of acute seizures with phenytoin. Patients with a previous history of seizures were excluded. Over a twenty two month period, we enrolled eight adults into the study.

As part of routine clinical monitoring of brain ECF biochemistry each patient had a commercially available microdialysis catheter (CMA 70, CMA/Microdialysis) with a 10 mm dialysis membrane length and 20 kDa molecular weight cut-off inserted into their brain tissue through a three divergent lumen, percutaneous, skull bolt (Technicam Ltd, Newton Abbott,

UK). Microdialysis catheter positioning followed the recommendations of the consensus meeting on microdialysis in neurointensive care[11]. The catheter was perfused with Perfusion Fluid CNS (CMA/Microdialysis), using a CMA 106 pump (CMA/Microdialysis), at a rate of 0.3 µL/min, and the sampling interval was 60 minutes. Microdialysate collected within the first four hours after catheter insertion was discarded to avoid insertion artefacts.

All subjects were treated with an initial intravenous loading dose of 15 mg/kg of phenytoin sodium (molecular weight 274.3 Da), followed by a daily oral phenytoin sodium dose of 300 mg, or equivalent dose of phenytoin liquid (molecular weight 252.3 Da), administered at 2200 hours, and commencing the day after the initial loading dose. In all subjects, the initial loading dose of phenytoin was administered at least 24 hours prior to the first blood and microdialysate sample collection. One subject (subject 2) received a second loading dose of 500 mg of phenytoin at 1900 hours on day 3 of blood and microdialysate sampling. None of the patients included in this study were receiving verapamil or probenecid which inhibit P-glycoprotein, and multi-drug resistance associated protein transporters, and might therefore affect neuronal phenytoin uptake[13].

Daily blood and microdialysate samples were taken at 0600 hours each morning. Samples were stored at -80^0C prior to assay. Plasma (300µl) was pipetted into the sample reservoir of an Amicon Centrifree micropartition system (Millipore, Hertfordshire, U.K.) in accordance with manufacturer's instructions, and centrifuged for 15 min at 3 000 g at a temperature setting of

25 ⁰C in a Sigma 2K15 refrigerated centrifuge (Sigma, Dorset, U.K.).
The concentrations of free phenytoin present in the ultrafiltrates and
microdialysate samples were determined by fluorescence polarisation
immunoassay using an Abbott TDx instrument and their reagents in
accordance with manufacturer's instructions (Abbott diagnostics, Maidenhead,
U.K.).

Lactate:pyruvate ratio (LPR) was calculated in hourly microdialysate samples
using the bedside CMA 600 analyser (CMA/Microdialysis). Daily blood and
microdialysate sampling was continued until invasive intracranial monitoring
was no longer clinically indicated and at that stage the microdialysis catheter
was removed.

Data were analysed using SPSS software (Version 11.0.1, SSPS Inc., Illinois,
USA). Correlation analysis was carried out using Pearson's Correlation
Coefficient with two tailed tests of significance, and p values <0.05 were
considered significant. The least square method was used for linear
regression analysis and the slope of the curve (β value) and standard error
(SE) were calculated to give an estimate of the biological relevance of the
findings. Assumptions required for linear regression were tested by assessing
the regression residuals for normality using the Shapiro-Wilk test.

Results

In Vitro Recovery

In Vitro Patient Study

Demographic information and details of presenting pathology are shown in table one. Timings of the insertion of the microdialysis catheter, and the initial blood and microdialysate sample collection for phenytoin assay, in relation to the time of injury are shown in table 2. Thirty one paired samples of plasma and microdialysate were analysed and the median number of paired samples per patient was 3 (range 2-9). Analysis of the residuals for each of the regression datasets presented showed no significant deviation from normality (Shapiro-Wilk test p>0.05) and residual mean close to zero (all less than 1×10^{-15}).

Figure 1A shows free phenytoin concentrations for the paired plasma/microdialysate samples classified by patient. As there was a variation in the number of paired samples per patient, each individual's mean [$Phen_{PLASMA}$] for the duration of microdialysate sampling was plotted against their respective mean [$Phen_{MD}$]. This allowed testing of the hypothesis that a linear relationship exists between these two variables for the group. This was not the case (figure 1B, r=0.34, p=0.41). There was also no correlation between mean microdialysate:plasma free phenytoin ratio and mean LPR (figure 2, r=0.11, p=0.79).

In two patients the period of clinical microdialysis monitoring produced a sufficient number of paired blood and microdialysate samples to allow for

intra-individual data analysis (patient one: nine paired samples and patient two: seven paired samples). In both cases a linear relationship between [$Phen_{PLASMA}$] and [$Phen_{MD}$] over the duration of the study was revealed. Results of linear regression analysis were r=0.92 (p<0.001), β value=0.31 (SE=0.05) for patient one (figure 3A), and r=0.88 (p<0.01), β value=0.38 (SE=0.09) for patient two (figure 3B).

Discussion

To our knowledge this is the first description of the measurement of [$Phen_{MD}$] in adults with acute brain injury. Our results suggest that, within the measured range, there is no general linear relationship between plasma and microdialysate free phenytoin concentration in patients with ABI. This implies that a fixed [$Phen_{PLASMA}$] target range for all patients, as is currently used, may not produce a predictable [$Phen_{ECF}$] and that conventional monitoring of [$Phen_{PLASMA}$] has limitations in this respect. However, in the two patients who allowed for individual data analysis a significant linear relationship was shown between [$Phen_{PLASMA}$] and [$Phen_{MD}$]. This suggests that, within a given individual, a constant ratio between [$Phen_{PLASMA}$] and [$Phen_{MD}$] may exist for a focal area of brain over time. The LPR indicates the extent of anaerobic metabolism in the tissue being monitored and is a measure of tissue ischaemia[14]. The lack of correlation between mean microdialysate:plasma free phenytoin ratio and LPR suggests that the discrepancy in microdialysate:plasma free phenytoin ratio across patients is not simply a result of reduced substrate delivery to this area of the brain. If this were the

case one might expect changes in LPR (reflecting oxygen delivery) to mirror changes in mean microdialysate:plasma free phenytoin ratio (reflecting phenytoin delivery). However LPR may be affected by mitochondrial dysfunction, leading to reduced oxygen utilisation despite adequate oxygen delivery, and changes in glucose availability. There are also obvious differences in the delivery mechanisms of oxygen and phenytoin and it is perhaps not surprising that we do not see a linear relationship between LPR and mean microdialysate:plasma free phenytoin ratio.

In this study we did not make a direct measurement of $[Phen_{ECF}]$ and the measured concentrations of $[Phen_{MD}]$ represent only a proportion of true $[Phen_{ECF}]$. This proportion is known as the relative recovery and is a consequence of the transit time of the perfusate along the semi-permeable microdialysis membrane being insufficient to allow complete equilibration between ECF and perfusate. Relative recovery of phenytoin has been investigated by several authors using a variety of catheter types, membrane lengths and perfusion rates[10,15,16]. However, it has not previously been reported for the commonly used catheter type and perfusion rate we describe in this study. We were not able to reproduce the results of one study showing a phenytoin relative recovery of 50% at a high flow rate of 1.65 µl/min[10].

Elucidation of the relative recovery of phenytoin is complicated by the fact that phenytoin may bind to the plastic tubing of the CMA 70 microdialysis catheter and this makes the calculation of absolute $[Phen_{ECF}]$ prone to error[15]. The binding of phenytoin to the microdialysis tubing will reduce the $[Phen_{MD}]$ and

thus the microdialysate:plasma free phenytoin ratio, but should not affect the coefficient of correlation between $[Phen_{MD}]$ and $[Phen_{PLASMA}]$. In addition, at constant perfusate flow rates and over the time course of this study we would expect phenytoin binding to the catheter to reach steady state.

There are two possible explanations for the lack of correlation between $[Phen_{PLASMA}]$ and $[Phen_{MD}]$ for our group data. Firstly, there may be real differences in individuals' free phenytoin concentration gradient between the plasma and the brain ECF. ABI comprises a range of pathology and causes widespread individual heterogeneity in the brain tissue, both in terms of cerebral blood flow and cell function[17]. This may cause disparities in the balance between delivery and uptake of antiepileptic drugs. The variable degree of blood brain barrier disruption that occurs after ABI may also affect the ECF:plasma free phenytoin ratio. Secondly, the relative recovery of phenytoin may vary between individuals. Relative recovery may be affected by focal anatomical variation surrounding the microdialysis catheter, the degree of tissue damage, and the length of time since catheter implantation. There is also likely to be some variation in relative recovery across a patient group and this may therefore contribute to the range of values of microdialysate:plasma free phenytoin ratio.

This study comprises a small number of subjects. Sample size calculations reveal that to detect a significant group correlation between $[Phen_{PLASMA}]$ and $[Phen_{MD}]$ of the strength we show in this study (r=0.34) would require approximately 60 subjects[18]. However an r value of 0.34 would correspond to

a wide scatter of data points and would still imply that a given serum free

phenytoin concentration would be a poor predictor of an individual's

$[Phen_{ECF}]$.

Conclusions

Our pilot data suggest that cerebral microdialysis is a technique that can

facilitate investigation of the neuropharmacokinetics of phenytoin at the

bedside. However, further studies are required to calculate the relative

recovery of phenytoin for an individual and to determine whether the use of

$[Phen_{ECF}]$ as a therapeutic target is able to improve seizure control after ABI.

Acknowledgements

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| Subject	Age	Sex	Pathology
1	53	Female	Aneurysmal Subarachnoid Haemorrhage
2	57	Female	Acute Subdural Haematoma
3	45	Male	Gunshot Head Wound
4	41	Male	Bilateral Traumatic Cerebral Contusions
5	16	Male	Diffuse Axonal Injury
6	50	Male	Acute Subdural Haematoma
7	38	Male	Extradural/Subdural Haematoma
8	47	Male	Acute Subdural Haematoma

Table 1: Demographic information and description of presenting injury for eight subjects in pilot study to investigate the relationship between brain extracellular fluid free phenytoin concentration and plasma free phenytoin concentration. Subjects 1 and 2 are those shown in figures 3A and 3B respectively.

| Subject	Day post injury of microdialysis catheter insertion	Day post injury of initial study sample collection
1	7	8
2	1	1
3	0	2
4	3	4
5	1	2
6	2	3
7	3	4
8	0	4

Table 2: Timings of the insertion of the microdialysis catheter, and the initial blood and microdialysate sample collection for phenytoin assay, in relation to the time of injury for eight subjects.
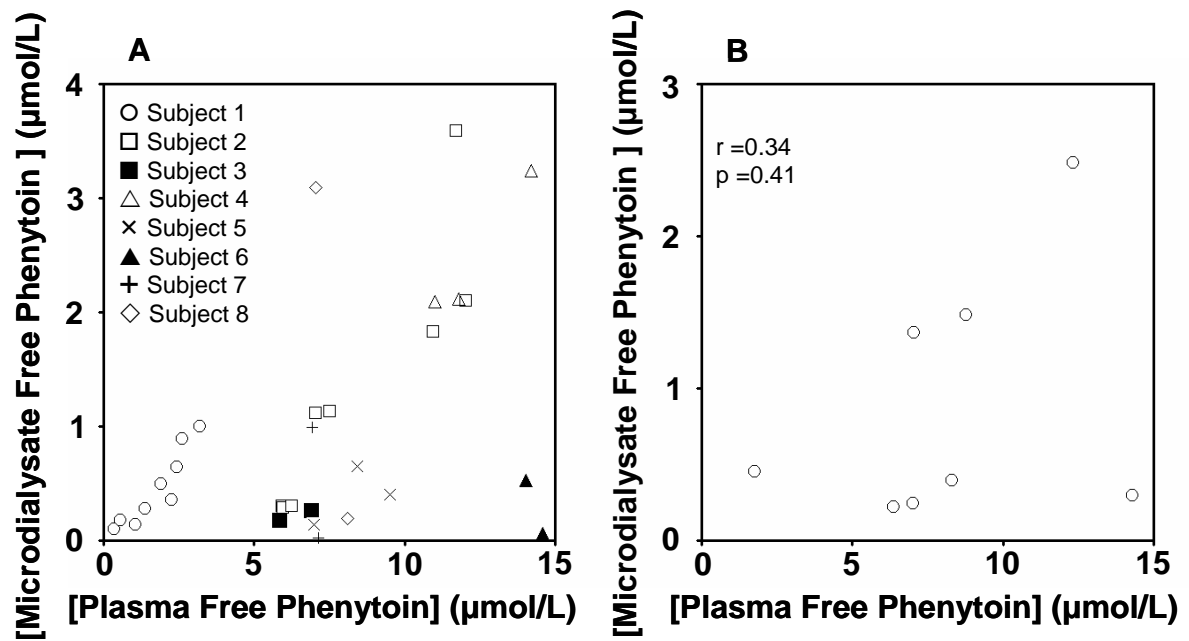
Figure 1. A: Microdialysate and plasma free phenytoin concentration for 31 paired samples from eight subjects, with subjects individually labelled. **B:** Mean data point plotted for each subject to allow assessment of correlation between microdialysate and plasma free phenytoin concentration for the group. Note there is no significant correlation (p=0.41).
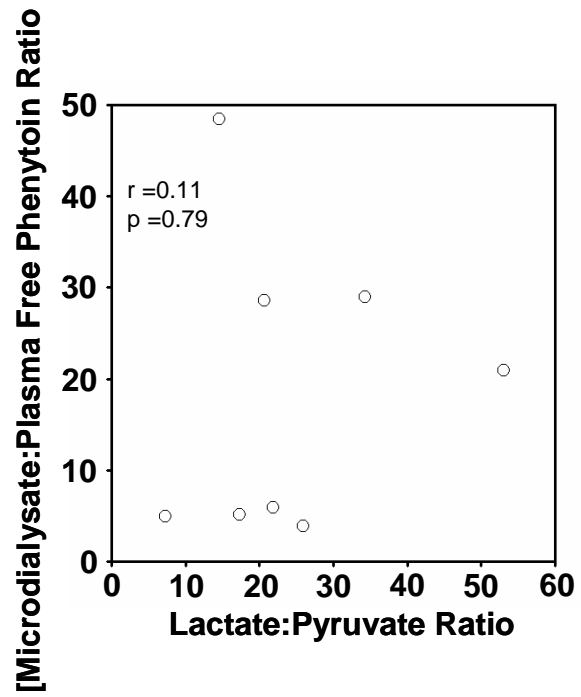
Figure 2: Mean microdialysate:plasma free phenytoin concentration ratio plotted against mean lactate:pyruvate ratio. Note there is no significant correlation (p=0.79).
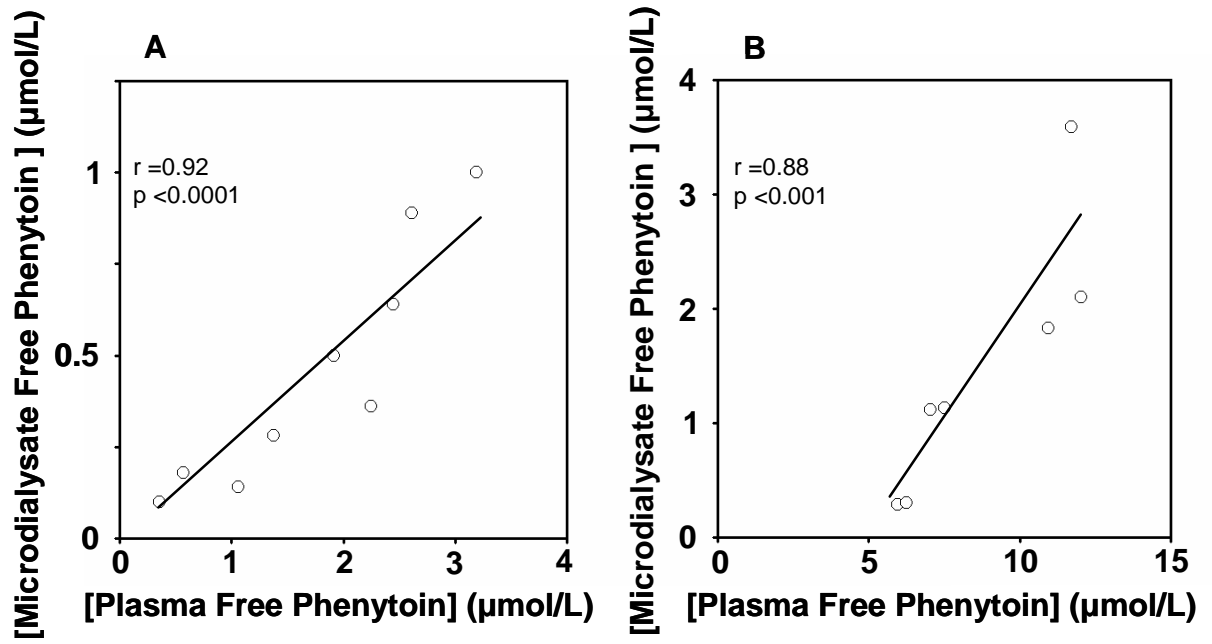
Figure 3. A: Nine paired microdialysate and plasma free phenytoin concentration results for subject one. Note significant correlation r=0.92 (p<0.001). **B:** Seven paired microdialysate and plasma free phenytoin concentration results for subject two. Note significant correlation r=0.88 (p<0.01).