PLASMA PROTEIN-BINDING AND CSF CONCENTRATIONS OF VALPROIC ACID IN MAN FOLLOWING ACUTE ORAL DOSING

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- 1 Simultaneous cerebrospinal fluid (CSF), total and free plasma valproic acid (VPA) concentrations were measured in 17 patients receiving two weight-adjusted VPA doses as seizure prophylaxis prior to diagnostic myelography or cisternography.
- 2 Free drug concentrations were similar when measured by equilibrium dialysis (ED) at 37°C for 24 h (Dianorm) or by a novel ultrafiltration (UF) method (EMIT freelevel system 1, SYVA) (ED: 2.3–35.5 mg⁻¹; UF: 1.3–33.6 mg⁻¹; $r = 0.78$, $P < 0.002$).
- 3 There was wide variation in total VPA concentration (39–154 mg⁻¹) and in free fraction (ED: 3.3–25.6%; UF: 5.9–24%). Concentration dependent protein binding was not demonstrated.
- 4 CSF VPA varied between 4.2 and 25.6 mg⁻¹ and was accurately reflected by free plasma VPA concentrations (ED: $r = 0.75$, $P < 0.005$; UF: $r = 0.93$, $P < 0.001$). CSF concentration also correlated with the total plasma VPA ($r = 0.76$, $P < 0.005$).
- 5 The Emit freelevel system 1 provides a rapid measure of unbound VPA in the plasma which may be suitable for routine clinical use.

Keywords valproic acid protein binding CSF concentration

Introduction

Valproic acid (VPA) is a branched-chain fatty acid with anticonvulsant properties which is widely used in the treatment of epilepsy. Its likely mechanism of action is augmentation of post-synaptic inhibition of gamma-aminobutyric acid mediated neurones (Spero, 1982). VPA is rapidly absorbed following oral administration and has a short elimination half-life (Gugler & Von Unruh, 1980). No direct relationship between total drug concentration and the clinical effect can be demonstrated (Schobben *et al.*, 1980; McQueen *et al.*, 1982). In common with other anticonvulsants, VPA is highly bound to serum proteins, mainly to albumin (Marty *et al.*, 1982). VPA exhibits concentration-dependent protein binding which partly accounts for considerable variability in the free-fraction and total plasma drug concentration (Bowdle *et al.*, 1981). Since the unbound drug is assumed to be responsible for its pharmacological and toxicological effects, the plasma measurement of free rather than total VPA has been encouraged (Levy, 1980). It has also been suggested that free drug concentration in the plasma approximates that in the cerebrospinal fluid (CSF) (Gugler & Von Unruh, 1980). We wish to

report the relationship of VPA concentration in the CSF to the free and total concentrations of the drug in the plasma following acute oral dosing.

Methods

Patients

Seventeen consecutive patients referred for neurological investigation were studied. Thirteen had full myelograms, the contrast being run up from the lumbar region, and four had a combined ventriculogram/basal cisternogram via the suboccipital route (Macpherson, 1980). Such patients are routinely given weight-related acute oral dosing with VPA as prophylaxis against convulsive seizures which may follow the introduction of water soluble contrast materials into the CSF. An initial dose based on 30 mg kg⁻¹ (40–55 kg: 1.5 g; 56–79 kg: 2 g; 71–85 kg: 2.5 g) is given at 20.00 h on the evening before the examination and a second dose of 15 mg kg⁻¹ (0.7 g; 1 g; 1.2 g) 1.5–2 h before the procedure. A third dose similar to the

second is given at 20.00 h in the evening after the examination. A small volume of CSF is routinely obtained for measurement of cellular and protein content prior to the introduction of contrast medium. CSF surplus to diagnostic requirement was made available to VPA measurement. Blood was withdrawn at the time of the contrast examination and immediately centrifuged. The plasma, together with 2 ml CSF, was stored at -20°C until analyses.

Clinical details of the patients together with CSF protein concentrations are shown in Table 1. Thirteen patients received no other drugs prior to the procedure. Three received analgesics (paracetamol, Distalgesic or zomepirac) and a further patient with hypopituitarism was taking cortisone acetate chronically as replacement therapy. In eight patients the myelogram was normal, in two there was moderate spondylosis and in two severe spondylosis with a significant but not complete hold-up to the flow of contrast. In one case a schwannoma was found which caused a complete obstruction (Table 1). This was the only patient in whom the CSF protein was elevated. The ventriculogram/cisternogram was normal in one patient, showed evidence of late post-haemorrhagic/surgical adhesions in one and in two demonstrated suprasellar mass lesions (a pituitary adenoma with suprasellar extension and a craniopharyngioma).

Analyses

Free plasma VPA was determined in duplicate using equilibrium dialysis (ED) and ultrafiltration (UF) methods. Dialysis was performed by isolating the cells

in a water bath at 37°C over 24 h (Dianorm). Plasma (0.9 ml) was injected into one chamber with a similar amount of phosphate buffer (0.13 M, pH 7.4) in the other. Free plasma concentration was calculated from the following equation:

$$f = \frac{\text{Cl (F)}}{\text{Cl (o)} - \text{Cl (F)}}$$

where f = free fraction

Cl (F) = final concentration of free ligand

Cl (o) = starting concentration of ligand

The ultrafiltration method utilised a novel filter system into which 1 ml plasma was injected (EMIT freelevel system 1, SYVA). The filter was spun at 1000–2000 g on an Heraeus Christ Minifuge GL at a temperature of 25°C for 60 min. A final ultrafiltrate volume of $> 200 \mu\text{l}$ was obtained for each sample.

Coefficient of variation was less than 10% for each method. All VPA measurements were carried out by enzyme immunoassay (EMIT, SYVA).

Statistical analysis was by the Spearman rank correlation procedure.

Results

Total and CSF VPA measurements in each dosage group are shown in Figure 1. As can be seen the majority of patients received a total of 3 g VPA while only four took the smaller and two the higher dose. There was no statistical correlation between dose or

Table 1 Clinical features and CSF protein levels in 17 patients undergoing diagnostic myelography or cisternography

Patient	Age (years)	Sex	Weight (kg)	Study	Diagnosis	Obstruction	CSF protein ($\text{g } 100 \text{ ml}^{-1}$)
1	44	M	83	M	Normal	—	0.35
2	42	M	65	C	Pituitary tumour	—	0.33
3	48	F	73	C	Adhesions	—	0.24
4	15	M	37	C	Craniopharyngioma	—	—
5	45	F	58	M	Normal	—	0.26
6	33	M	65	M	Schwannoma	+	3.35
7	53	F	61	M	Spondylosis	—	0.45
8	17	F	56	M	Normal	—	0.39
9	57	M	54	M	Spondylosis	—	0.45
10	55	M	68	C	Normal	—	0.19
11	43	F	60	M	Normal	—	0.46
12	32	F	61	M	Normal	—	0.30
13	50	M	86	M	Normal	—	0.75
14	35	F	36	M	Normal	—	0.28
15	28	M	56	M	Spondylosis	±	0.82
16	58	M	57	M	Spondylosis	±	0.37
17	55	M	68	M	Normal	—	0.45

+ total spinal obstruction ± partial 'hold-up' — no obstruction

M = Myelogram C = Cisternogram

Figure 1 Total plasma VPA (○) and CSF VPA (●) concentrations for the three groups of patients receiving different doses of the drug prior to neuroradiological examination.

weight and total or CSF VPA concentrations. There was substantial variation in total plasma ($39\text{--}154\text{ mg l}^{-1}$), CSF ($4.2\text{--}25.6\text{ mg l}^{-1}$) and free plasma (ED: $2.3\text{--}35.5\text{ mg l}^{-1}$; UF: $1.3\text{--}33.6\text{ mg l}^{-1}$) VPA concentrations despite ingestion of similar weight-adjusted doses. The free fraction varied from 3.3–25.6% by ED and 5.9–24% by UF. Correlation of total VPA concentration with free fraction by both methods did not attain statistical significance (ED: $r = 0.38$; UF: $r = 0.35$).

Free VPA concentrations were similar whether

Figure 2 Correlation between free plasma valproic acid (VPA) concentrations measured by equilibrium dialysis (Dianorm) and ultrafiltration (EMIT freelevel system 1, SYVA) techniques in 17 patients receiving prophylactic VPA prior to myelography or cisternography. $r = 0.78$, $P < 0.002$.

Figure 3 Correlation between CSF valproic acid (VPA) concentration and free plasma VPA using an ultrafiltration system in 17 patients receiving prophylactic VPA prior to myelography or cisternography. $r = 0.934$, $P < 0.001$.

measured by ED or UF ($r = 0.78$, $P < 0.002$; Figure 2) but UF correlated better with CSF concentrations ($r = 0.93$, $P < 0.001$; Figure 3) than ED ($r = 0.75$, $P < 0.005$; Figure 4). Total VPA concentrations also correlated with CSF VPA ($r = 0.76$, $P < 0.005$; Figure 5) and free concentrations by both methods ($r = 0.79$, $P < 0.002$ for both). The patient with very high CSF protein concentration and total spinal obstruction (Patient 6 on Table 1) received 3 g VPA prior to the study. He had a total VPA concentration of 91 mg l^{-1} with CSF concentration of 19 mg l^{-1} . As can be seen from Figure 1, his results were not unusual for the group. Free plasma VPA was lower than in CSF (ED: 9.3 mg l^{-1} ; UF: 11.2 mg l^{-1}) and this may indicate binding of the drug to CSF protein.

Figure 4 Correlation between CSF valproic acid (VPA) concentration and free plasma VPA measured following equilibrium dialysis in 17 patients receiving prophylactic VPA prior to myelography or cisternography. $r = 0.745$, $P < 0.005$.

Figure 5 Correlation between CSF valproic acid (VPA) and total plasma VPA concentrations in 17 patients receiving prophylactic VPA prior to myelography or cisternography. $r = 0.76$, $P < 0.005$.

Discussion

Free plasma VPA concentrations were similar when measured by equilibrium dialysis and the new ultrafiltration technique linked to an enzyme immunoassay system (EMIT freelevel system 1). Although all methods for measuring protein binding have their drawbacks, equilibrium dialysis is most often employed and may be regarded as the 'gold standard' for such methodology (Rowland, 1980). For VPA binding at least 12 h dialysis is required at a temperature of 37°C (Gugler & Mueller, 1978). For convenience, dialysis was carried out for 24 h in this study. Free concentrations were available using the new ultrafiltration system linked to the EMIT spectrophotometer within 2 h of sample collection. This system, therefore, may be ideal for routine measurement of free VPA concentration in the clinical setting as it

may avoid the *in vitro* changes due to free fatty acid release following prolonged dialysis (Albani *et al.*, 1983).

Free plasma VPA levels closely reflected that found in the CSF and this is in keeping with similar findings for carbamazepine and phenytoin (Bonati *et al.*, 1982) and for VPA in the rhesus monkey (Levy, 1980). In this acute study total VPA concentrations also correlated with CSF levels and no evidence of dose-dependent binding was found as clearly occurs in the steady state situation (Bowdle *et al.*, 1981). This may be due to the short duration of therapy or the relatively small number of patients studied. Total VPA concentrations show a poor correlation with clinical efficacy or toxicity (Schobben *et al.*, 1980) which may, in part, relate to the variability in VPA free fraction (Levy, 1980), although a non-reversible effect of VPA has also been suggested (Rowan *et al.*, 1979). Further studies will be required to define the relationship between free drug concentration, therapeutic effect and clinical intoxication.

There was substantial variation in VPA concentrations in blood and CSF despite a similar weight-related dose in a group of patients who did not have systemic disease. As sodium valproate is rapidly and completely absorbed, this must reflect variation in distribution and metabolism of the drug (Gugler & Von Unruh, 1980). Nevertheless this study indicates that total plasma VPA may provide some indication of the CSF concentration following acute oral VPA dosing. However, controlled studies of efficacy must be carried out before this regime can be recommended for routine use as seizure prophylaxis during invasive neurosurgical procedures.

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