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DECREASED PLASMA PROTEIN BINDING OF PHENYTOIN IN PATIENTS ON VALPROIC ACID

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1 Plasma protein binding of phenytoin and of valproic acid were measured in ten epileptic patients on this drug combination. Ten other epileptics not on valproic acid served as controls. All patients had normal kidney function.

2 The measured free fraction of phenytoin among the patients on valproic acid ranged from 12.5 to 23.2% and after recalculation to a plasma albumin level of 45 g/l from 12.5 to 20.0 (median 15.4%). This differed significantly (P = 0.002, Mann- Whitney U-test) from the control patients where the *normalized* values ranged from 9.9 to 13.9% with a median value of 11.8%.

3 The measured free fractions of phenytoin and of valproic acid showed a significant correlation which, however, was due to the quantitative relation between the degree of binding of both these drugs and the concentration of plasma albumin. There was no discernable relation in this material between the free concentration of valproic acid and the free fraction of phenytoin.

4 It is concluded that patients on combined treatment with phenytoin and valproic acid have an unpredictably raised free fraction of phenytoin. This drug interaction therefore can complicate the important plasma level monitoring of phenytoin in epileptic patients unless the free concentration of this drug can be analysed or estimated.

Introduction

Monitoring of plasma levels of antiepileptic drugs now plays an important role in optimizing the treatment of epileptic patients (see e.g. Lund, 1974; Kutt & Penry, 1974; Eadie, 1976). This is especially true for phenytoin by virtue of its narrow therapeutic range and concentration-dependent kinetics.

Most laboratories measure the concentration of total drug in plasma on the premise that inter-individual differences in plasma protein binding are small. This has been documented for phenytoin provided that kidney function and albumin levels are normal (Lunde, Rane, Yaffe, Lund & Sjöqvist, 1970; Barth, Alván, Borga & Sjöqvist, 1976). Other antiepileptic drugs, not including valproic acid were shown not to interact with phenytoin binding (Lunde *et al.*, 1970). Evidence that valproic acid ((dipropyl-acetic acid) displaces phenytoin from its binding sites in plasma has been provided in animal experiments (Patsalos & Lascelles, 1977). Also, the *in vitro* binding of phenytoin added to human plasma is decreased by the addition of valproic acid (Jordan, Shillingford & Steed, 1976; Patsalos & Lascelles 1977; Monks, Boobis, Wadsworth & Richens, 1978). Such an interaction may be pronounced because of the high (about 90%) degree of plasma protein binding of valproic acid (Gugler, Schell, Eichelbaum, Fröscher & Schulz, 1977; Wulff, Flachs, Würtz-Jörgensen & Gram, 1977; Klotz, Rapp & Müller, 1978; Gugler & Mueller, 1978) and the high molar concentrations of valproic acid (up to 900 μmol/l) observed at therapeutic doses (Schobben, van der Kleijn & Gabreals, 1975). Recent detailed *in vitro* investigations on this interaction have documented the competitive displacement of phenytoin bound to plasma protein by concentrations of valproic acid above 280 μmol/l (Monks *et al.*, 1978).

Bardy, Hari, Lehtovaara & Majuri (1976), Mattson, Cramer, Williamson & Novelly (1978), Adams, Luders & Pippenger (1978) and Wilder, Willmore, Bruni & Villarreal (1978) observed lower total serum levels of phenytoin when their patients simultaneously were treated with valproic acid. In the study by Mattson *et al.* (1978) the valproic acid induced fall in total plasma concentration of phenytoin was shown to occur parallel to a rise in the free fraction of phenytoin while free concentrations in plasma were constant or moderately increased. The effects of valproic acid on the degree of plasma binding and the total plasma concentrations of phenytoin could furthermore be demonstrated to be dose-related (Mattson *et al.*, 1978).

The objective of our study was to examine to what extent valproic acid may affect the unbound fraction of phenytoin in the clinical situation, i.e. in patients of

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various ages, various doses of valproic acid and in most cases unknown compliance of the two drugs. We also wanted to study whether the displacement effect of valproic acid could be predicted from simultaneously determined valproic acid levels. We found that unbound (pharmacologically active) phenytoin levels can not be predicted from valproic acid levels, but will have to be determined routinely with direct methods in patients treated with this drug combination.

Methods

Plasma samples from ten epileptic patients on the combined treatment of phenytoin and valproic acid were analysed. The doses of these drugs had been unchanged for at least two weeks prior to the blood sampling and the sample taken before the morning dose. The patients, all of whom were hospitalized, had various forms and degrees of severity of epilepsy and various underlying disorders. Most patients were also treated with other drugs (Table 1). According to

Table 1 Concomitant maintenance drugs in patients treated with valproic acid and phenytoin

Patient number	*Age (years)*	*Other drug(s)*	*Daily dose*
1	9	Carbamazepine	0.9 g
2	8	Carbamazepine	0.4 g
3	12	Phenobarbitone	15 mg
4	9	None	
5	9	None	
6	10	Carbamazepine	0.8 g
7	10	Clonazepam	0.25 mg
8	8	None	
9	22	Carbamazepine, clonazepam	0.6 g 5 mg
10	24	Ethosuximide	0.75 g

the clinical history and standard biochemical blood tests all patients had normal kidney function. For comparison plasma samples from ten epileptic patients treated with phenytoin but not with valproic acid were analysed in parallel.

Phenytoin plasma concentrations were determined by gas chromatography (Orme, Borgå, Cook & Sjöqvist, 1976). The unbound fractions of phenytoin and valproic acid were determined simultaneously by equilibrium dialysis for 4 h at 37°C using the method of Ehrnebo, Agurell, Jalling & Boréus (1971) after addition of trace amount of [^{14}C]-phenytoin (Radiochemical Centre, Amersham, Buckinghamshire, England).

The isotope was purified by preparative liquid chromatography on a Micro Bondapak C-18 column, 30 cm by 4 mm i.d. (Waters Ass. Inc.) using as mobile phase a 38:62 (v/v) mixture of acetonitrile and water. Valproic acid in plasma was determined with gas chromatography using the method of Jakobs, Bojasch & Hanefeld (1978). The measurement of the unbound concentration of valproic acid in the equilibrium dialysis chambers required a more sensitive analytical technique than that by Jakobs *et al.* (1978). This requirement was met by a slight modification of the gas chromatographic method by Schulz & Toseland (1977). Albumin concentrations were determined by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using M-Partigen plates (Behringwerke AG, Marburg, West Germany).

Recalculation of experimental binding data to a *normalized* plasma albumin concentration of 45 g/l was performed by the formula given by Odar-Cederlöf & Borgå (1976).

Results

The percentage unbound phenytoin in serum ranged from 12.5 to 23.2 (median 14.9) among the patients concomitantly treated with valproic acid (Table 2). Recalculation of these values to the binding capacity at the plasma albumin concentration of 45 g/l yielded a range of values from 12.5 to 20.0 with a median value 15.4% (Figure 1, Table 2). The corresponding corrected values for the control patients were 9.3–13.9 with a median of 11.8% (Figure 1, Table 3). The difference in free fraction of phenytoin between the two groups of patients was statistically significant ($P = 0.002$, Mann-Whitney U-test).

The unbound fraction of valproic acid in plasma ranged from 6.4 to 28.7 (median 7.7)% and after correction to 45 g/l of albumin in plasma, 6.7–20.0 (median 8.4)%. One patient had an extremely high unbound fraction of valproic acid (No 1, with Gaucher's disease). When this patient was omitted from the material the *mean* unbound fraction of the nine remaining patients was 8.5% and the range 6.7–11.2%.

Uncorrected free fractions of phenytoin and valproic acid correlated ($r = 0.79$; $P < 0.01$) which to a large extent depended on the high free fractions in patient No 1. Exclusion of this patient markedly weakened the apparent correlation ($r = 0.55$; $P > 0.05$). As expected, there was a significant correlation between the plasma albumin concentration and the uncorrected unbound fractions of phenytoin ($r = 0.68$; $P < 0.05$) and of valproic acid ($r = 0.86$; $P < 0.01$). After correction of the binding values to the same plasma albumin concentrations (45 g/l) the binding of phenytoin and valproic acid showed no correlation at all ($r = 0.06$).

Within the range of total plasma concentrations of valproic acid in this study (87–269 μmol/l) no relation

Table 2 Patients on valproic acid. Plasma concentrations of albumin, creatinine or urea, phenytoin and valproic acid and free fractions of phenytoin and valproic acid

Patient number	*Sex*	*Age (years)*	*Albumin (g/l)*	[1]*Creatinine (μmol/l)*	[2]*Urea (mmol/l)*	*Phenytoin* *Total concentration (μmol/l)*	*Free fraction (%)*	**Free fraction (%) (normalized)*	*Valproic acid* *Total concentration (μmol/l)*	*Free fraction (%)*	**Free fraction (%) (normalized)*
1	M	9	28	30		60	23.2	15.8	171	28.7	20.0
2	M	8	30		3.8	85	18.5	13.1	118	15.9	11.2
3	M	12	43		3.5	49	14.0	13.5	151	7.0	6.7
4	F	9	38		3.2	65	14.3	12.4	124	12.3	10.6
5	M	9	53			80	15.3	17.5	202	7.7	9.0
6	M	10	45	33		35	12.5	12.5	143	7.7	7.7
7	F	10	41	32		60	21.5	20.0	87	10.5	9.7
8	M	8	49		3.8	73	15.2	16.3	269	6.4	6.9
9	F	22	50	70		35	13.7	15.0	134	6.7	7.4
10	F	24	50	68		94	14.5	15.9	255	6.6	7.3
Median (Range)		9.5 (8–24)	44 (28–53)			62.5 (35–94)	14.9 (12.5–23.2)	15.4 (12.5–20.0)	147 (87–269)	7.7 (6.4–28.7)	8.4 (6.7–20.0)

**Normalized* to a plasma albumin level of 45 g/l according to Odar-Cederlöf & Borga (1976)
[1]Reference values < 120 μmol/l
[2]Reference values 3.0–7.5 mmol/l

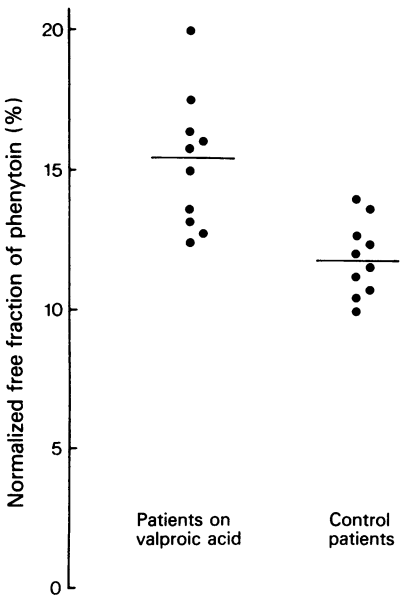

Figure 1 Free fraction of phenytoin in patients treated with valproic acid ($n = 10$) and in control patients ($n = 10$). Individual values and medians are shown. All values are *normalized* to the same binding capacity, namely a plasma albumin concentration of 45 g/l.

between the level of valproic acid and the unbound fraction of phenytoin was observed. The actual *unbound* concentration of valproic acid on the other hand showed a weak correlation ($r = 0.65$; $P < 0.05$) with the unbound fraction of phenytoin. Again, *normalizing* the phenytoin binding values to an albumin level of 45 g/l eliminated this apparent correlation ($r = 0.03$).

Discussion

The absolute figure of protein binding of a drug is dependent on the technique used and varies with temperature, pH and drug concentration. The degree of protein binding of phenytoin in our control material is in the range previously reported under similar experimental conditions (Lunde *et al.*, 1970; Odar-Cederlöf & Borgå, 1976). Also the fractional binding in plasma of valproic acid (patient No. 1 excluded) corresponds well with data from other investigators (Gugler *et al.*, 1977; Gugler & Mueller, 1978). By comparing total levels and plasma binding of phenytoin in the same individuals before and during valproic acid co-medication, Mattson *et al.* (1978) were the first to demonstrate the effect of valproic acid to decrease plasma binding of phenytoin in patients. In our study a significantly higher free fraction of phenytoin was found for the valproic acid patient group. The difference remained after correction of the binding values to the same albumin concentration for all patients. The average total plasma concentration of phenytoin was somewhat higher in the valproic acid patient group than in the control material. This should not however influence the results since the free fraction of phenytoin is constant at levels below 100 μmol/l (Lunde *et al.*, 1970; Odar-Cederlöf & Borgå, 1976).

Competition between phenytoin and valproic acid for a common binding site on the albumin molecule was recently documented (Monks *et al.*, 1978). According to accepted theory (Edsall & Wyman, 1958) the binding of phenytoin would be expected to decrease proportionally to the concentration of *unbound* valproic acid. A weak correlation was observed only when related to uncorrected binding

Table 3 Control material. Patients not on valproic acid. Plasma concentrations of albumin and phenytoin and free fractions of phenytoin

Patient number	*Sex*	*Age (years)*	*Albumin (g/l)*	*Phenytoin* *Total concentration (μmol/l)*	*Free fraction (%)*	**Free fraction (%) (normalized)*
1	F	47	43	51	11.7	11.2
2	M	46	47	59	10.3	10.7
3	M	72	34	25	12.7	9.9
4	F	4	44	51	12.8	12.6
5	F	14	49	38	11.4	12.3
6	F	6	49	16	12.9	13.9
7	F	12	40	15	13.2	11.9
8	F	10	47	55	11.2	11.6
9	F	6	47	39	13.1	13.6
10	M	8	47	12	10.1	10.5
Median (Range)		11 (4–72)	47 (34–49)	38.5 (12–59)	12.2 (10.1–13.2)	11.8 (9.9–13.9)

**Normalized* to a plasma albumin level of 45 g/l according to Odar-Cederlöf & Borga (1976).

values indicating, as previously known, that the albumin concentration is an important variable for the binding in plasma of both valproic acid and phenytoin.

Carbamazepine, ethosuximide and phenobarbitone have previously been shown not to interfere with the plasma binding of phenytoin (Lunde *et al.*, 1970). The parallel use of these drugs therefore will not affect the interpretation of valproic acid as the displacing agents with regard to phenytoin binding.

The results from this study confirm previous more extensive studies *in vitro* (Monks *et al.*, 1978) and *in vivo* (Mattson *et al.*, 1978) showing that patients on a combined treatment with phenytoin and valproic acid should be expected to have an elevated unbound fraction of phenytoin in plasma. Mattson *et al.* (1978) found that the addition of valproic acid to the phenytoin medication caused total phenytoin levels to decrease while unbound levels were essentially constant. These authors therefore conclude that 'unless the total phenytoin is low the clinical effect should be insignificant'.

However our starting-point is quite different since like many other laboratories we perform anti-epileptic drug plasma level monitoring, including determinations of total plasma levels of phenytoin and valproic acid. In the practical situation, when a plasma sample is sent to us for analysis, we may or may not have a phenytoin level obtained before valproic acid medication is introduced. We usually know the prescribed doses of the two drugs, but we have no means of assessing compliance, nor can we assume that phenytoin compliance is constant after putting the patient on valproic acid. Thus a pharmacokinetic service laboratory such as ours is left with only one reliable source of information, namely the investigated plasma sample. Our study shows that the unbound phenytoin concentration can not be predicted from the total phenytoin level, knowing the simultaneous valproic acid level. Thus to provide meaningful phenytoin level data to the prescribing physician, the unbound drug fraction should be routinely determined in the plasma sample by a direct method, such as equilibrium dialysis or ultrafiltration.

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