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Interaction between vigabatrin and phenytoin

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1 The study was designed to determine the mechanism by which vigabatrin causes a fall in plasma phenytoin concentrations when added to the drug therapy of eight epileptic patients.
2 Total plasma phenytoin concentration was measured before and at intervals during 5 weeks' treatment with vigabatrin.
3 Plasma protein binding of phenytoin, the urinary ratio of phenytoin to 5-(*p*-hydroxyphenyl)-5-phenylhydantoin, and antipyrine clearance were measured before and at the end of treatment period.
4 Mean plasma phenytoin concentration fell significantly by 23% during the fifth week.
5 No change was found in any of the other measures.
6 Although an interaction between phenytoin and vigabatrin has been confirmed, the mechanism has not been elucidated.

Keywords vigabatrin phenytoin interactions protein binding

Introduction

Interactions between antiepileptic drugs are common (Perucca & Richens, 1985). Sometimes these interactions may cause an increase in the therapeutic or toxic effects of a drug, for example, the potent inhibitory effect of sulthiame on phenytoin metabolism. On the other hand, hepatic enzyme induction by phenytoin, carbamazepine, phenobarbitone or primidone may reduce the effect of an added drug such as sodium valproate. During the early clinical development of a new antiepileptic drug it is important to investigate the possibility of interactions because initial trials are usually undertaken in patients who are already receiving conventional drugs but who continue to experience seizures despite the therapy. Inhibition of the metabolism of an existing drug by the new drug may confound the interpretation of a reduction in seizures or appearance of an adverse event. Conversely, the new drug may appear to be ineffective if its metabolism is greatly enhanced by enzyme induction.

Two early trials of vigabatrin demonstrated a significant fall in plasma phenytoin concentration following introduction of the new drug. In a double-blind placebo-controlled study, Rimmer & Richens (1984) found that the mean serum phenytoin level in a group of ten patients receiving this drug fell significantly from 59 ± 30 μmol l^{-1} to 40 ± 13 μmol l^{-1} on addition of 1.5 g of vigabatrin twice daily compared with the period during which these patients were receiving placebo. Browne *et al.* (1987) found a reduction of 20% in serum phenytoin levels in a single-blind multicentre study. Phenobarbitone and primidone concentrations were also significantly reduced. No attempt was made in either of these trials to elucidate the mechanism of the interactions. Fortunately, the change in phenytoin levels was in a direction that did not confound the interpretation of the trials because, despite causing a fall in phenytoin concentrations, vigabatrin nevertheless reduced seizure frequency. The aim of the study described here was to

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characterize the mechanism by which vigabatrin reduces plasma phenytoin levels. The possibilities investigated were (i) a reduction in absorption, (ii) an increase in metabolism and (iii) displacement from plasma protein binding sites.

Methods

Patients

Eight epileptic outpatients aged between 17 and 51 years were recruited into the study. All of them had chronic drug-resistant epilepsy and were taking phenytoin as part of their usual anticonvulsant therapy. The dose of phenytoin had not been altered for 1 month prior to the start of the study and remained unaltered throughout. None of the patients had any other significant medical disorder and biochemical and haematological parameters were all normal. Clinical details of the patients are summarized in Table 1. All gave informed written consent.

Study design

In order to document any changes in plasma phenytoin concentration during vigabatrin therapy regular blood sampling for anticonvulsant drug analysis was performed before, during and after a period of vigabatrin therapy. Blood samples were obtained between 09.00 and 10.00 h on each occasion. At least two baseline antiepileptic drug levels were measured in each patient, 1 week apart before starting vigabatrin. A plasma sample to determine plasma protein binding of phenytoin was also obtained and a 24 h urine sample collected for measurement of the ratio of the major metabolite of phenytoin, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin to the parent drug (*p*-HPPH:DPH ratio). As a test of liver enzyme induction, an antipyrine test was performed prior to commencing vigabatrin.

Vigabatrin was then added on to the usual medication of the epileptic patients, initially in a dose of 1 g twice daily, increasing after 1 week to 1.5 g twice daily. The dose was continued for 4 weeks and then the various tests were repeated, including phenytoin binding, urine *p*-HPPH:DPH ratio and antipyrine test. The dose of vigabatrin was then reduced to 2 g daily and stopped after a week. Compliance with medication was encouraged by the use of 'Dosette' tablet containers and medication records. Throughout the study the patients kept seizure records and noted any adverse events. Ethical approval was obtained from the hospital's Ethics Committee.

Table 1 Clinical details of patients

Patient	*Age (years)*	*Sex*	*Seizure type*	*Seizure frequency*	*Antiepileptic medication*
01	26	F	CPS with 2° generalisation	4 per month	DPH 300 mg CBZ 800 mg Clobazam 20 mg
02	38	M	CPS with 2° generalisation	6 per month	DPH 300 mg CBZ 1200 mg Clobazam 30 mg
03	31	M	CPS with 2° generalisation	6 per month	DPH 150 mg CBZ 800 mg SV 2g
04	51	M	CPS with 2° generalisation	8 per month	DPH 400 mg SV 1500 mg
05	40	M	CPS with 2° generalisation	4 per month	DPH 300 mg CBZ 1000 mg Clobazam 20 mg
06	27	F	Tonic-clonic convulsions	3 per month	DPH 180 mg CBZ 800 mg
07	17	M	CPS	4 per month	DPH 350 mg SV 2g
08	17	M	CPS	8 per month	DPH 300 mg CBZ 800 mg

CPS complex partial seizures, DPH phenytoin, CBZ carbamazepine, SV sodium valproate.

Antiepileptic drug assay

Plasma samples were stored at −20° C until assayed for antiepileptic drugs in a single batch using a Fluorescence Polarisation Immunoassay (TDX-Abbot) (Wang & Peter, 1985).

Antipyrine tests

Antipyrine has long been used as a model to study the induction or inhibition of oxidative drug metabolising enzymes (Vessel, 1979; Park, 1982). It is suitable because it is rapidly and completely absorbed from the gastrointestinal tract and distributed in total body water with only a small degree of tissue and plasma protein binding. It is metabolised almost completely with negligible renal elimination of unchanged drug. Because of the low pKa (1.4) of antipyrine and its small degree of plasma protein binding, saliva antipyrine concentrations are closely related to plasma concentrations. It is possible, therefore, to predict plasma antipyrine kinetics from saliva samples, which are much more acceptable to the patients than repeated venepuncture (Fraser *et al.*, 1976). Even in epileptic patients who are enzyme-induced, the bioavailability of oral antipyrine is essentially complete with negligible presystemic elimination in gut or liver (Rimmer *et al.*, 1986).

The antipyrine test was carried out as follows: Antipyrine 600 mg dissolved in 100 ml of water was administered orally after an overnight fast. Mixed stimulated saliva samples were obtained at times 0, 1, 2, 3, 5, 8, 24 and 32 h. The saliva samples were stored frozen at −20° C until analysed by high performance liquid chromatography (Greenblatt *et al.*, 1983). Within batch coefficient of variation of the assay was less than 5%. The antipyrine half-life was then calculated for each subject using all the data points after 2 h, thus excluding the distribution phase. Area under the plasma concentration-time curve was calculated using the trapezoidal method and then antipyrine clearance was calculated by dividing the dose administered by the area under the curve.

Measurement of urinary p-*HPPH:DPH ratio*

Less than 5% of the daily dose of phenytoin is excreted unchanged in the urine (Bochner *et al.*, 1973; Karlen *et al.*, 1975). About 70–80% is eliminated as *p*-HPPH, mainly in the conjugated form. The ratio of metabolite to parent drug is determined both by the genetic drug metabolising capacity of an individual as well as the steady state plasma concentration of the parent drug (Houghton & Richens, 1974b). As the latter rises the hydroxylation mechanism becomes saturated: *p*-HPPH production fails to rise in proportion and therefore relatively more parent drug appears in the urine. The *p*-HPPH:DPH ratio can be used as an estimate of the rate of hydroxylation of phenytoin by hepatic enzymes. A decrease in the ratio occurs when phenytoin metabolism is inhibited, e.g. with sulthiame (Houghton & Richens, 1974a) and an increase in the ratio would be expected if phenytoin metabolism is induced.

Because the *p*-HPPH is present in urine mainly in the conjugated form, the urine samples were incubated with β-glucuronidase (1000 U to 200 μl urine) for 24 h at 37° C to break the glucuronide conjugate prior to assay. An h.p.l.c. method was used to measure the concentrations of phenytoin and *p*-HPPH in the urine samples. After extraction with ethyl acetate, chromatography was performed using a 5 μm spherisorb ODS column and ultraviolet detection at 215 nm. 5-(*p*-hydroxyphenyl)-5-*p*(tolyl)-hydantoin was used as the internal standard. The coefficient of variation of the assay was less than 10%.

Plasma protein binding

This was determined after equilibrium dialysis (using a commercial apparatus, Dianorm—cell size 1 ml) against a phosphate buffer (pH 7.4) at 37° C for 3 h after the addition of trace amounts of [^{14}C]-phenytoin (Amersham) to the patients' plasma samples. Radioactivity was determined using a liquid scintillation counter and quench correction was performed using the external standards ratio method. The free fraction was calculated by dividing the disintegrations on the buffer side by the disintegrations on the plasma side. The pH of the plasma samples at the end of dialysis was never greater than 7.5. There was less than 2% non-specific binding to the dialysis membrane (Spectrapor II [Spectrum Medical Industries Inc., Los Angeles, U.S.A.]—molecular weight cut-off 12,000), and no detectable leakage of protein across the membrane (minimum level of detection = 2 g l^{-1}). Purity of the [^{14}C]-labelled phenytoin was greater than 98% before and after dialysis. The dilutional effect at the end of dialysis was estimated to be 8% by measuring the albumin concentration of the plasma before and after dialysis. The coefficient of variation of this method for duplicate samples = 3.28%.

In vitro *binding studies*

Phenytoin was added to plasma from a volunteer to give a concentration of 60 μmol l^{-1} (the

middle of the therapeutic range) and a small amount of [^{14}C]-phenytoin was also added (2.2 μmol l^{-1}). Then vigabatrin was added to half the plasma to give a concentration of 150 μmol l^{-1}. Five equilibrium dialysis cells were filled with plasma containing phenytoin alone and five were filled with plasma containing phenytoin and vigabatrin. All ten cells were dialysed against phosphate buffer solution for 3 h at 37° C, as described above.

Results

Effects of vigabatrin on seizure frequency

Although the period of vigabatrin administration was short and it was not given double-blind with a placebo period, at least four out of the eight patients appeared to show a useful therapeutic response to vigabatrin. The most common adverse events noted by the patients were drowsiness and dizziness although in none of the patients were the symptoms severe.

Plasma phenytoin concentrations

A mean baseline phenytoin concentration was calculated for each patient by taking the mean of at least four values, two obtained prior to starting vigabatrin and two post-study samples, taken 4 or more weeks after stopping vigabatrin. The phenytoin concentrations were unaltered compared with baseline values until the fourth week of full dose vigabatrin therapy (Figure 1), when a drop in plasma phenytoin occurred in all eight patients, the mean decrease in plasma phenytoin

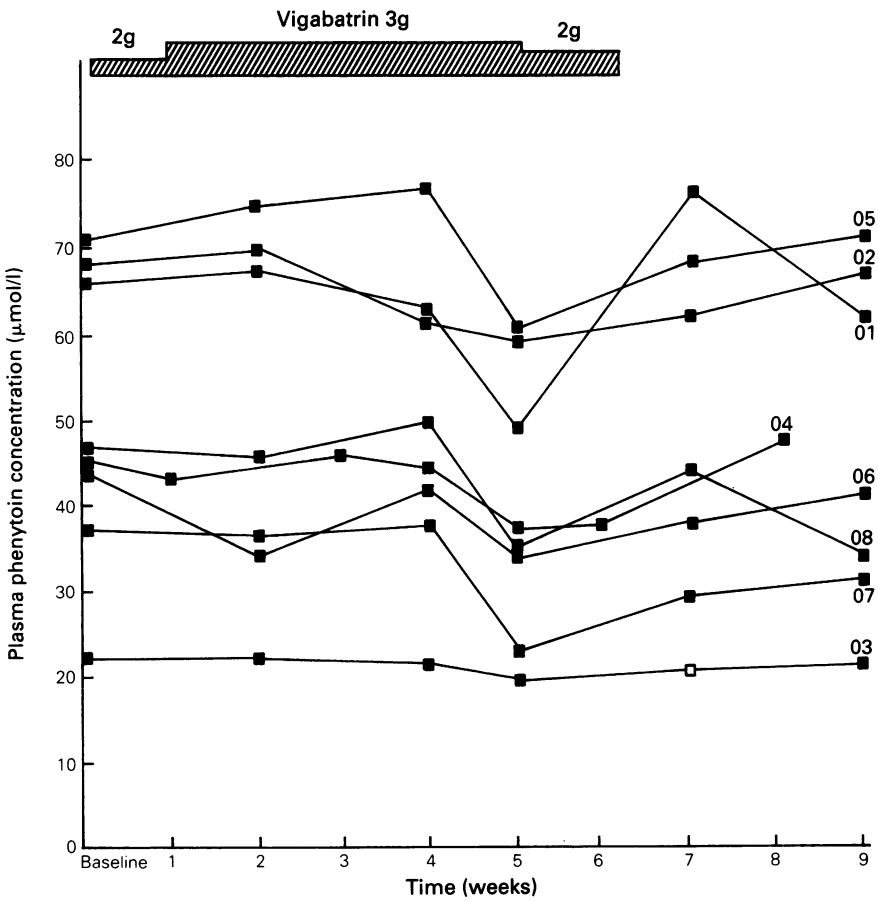

Figure 1 Plasma concentrations of phenytoin (μmol l^{-1}) in eight patients before, during and after vigabatrin therapy. The values on week 5 are significantly lower than those measured in the previous weeks (two-tailed paired Student's *t*-test, $t = 7.33$, $P < 0.001$).

= 23.4 ± 9.03% (mean ± s.d.) (two-tailed paired Student's *t*-test, $t = 7.33$, $P < 0.001$). One week after stopping vigabatrin, the phenytoin concentrations had risen towards the baseline values and by 4 weeks after ceasing vigabatrin therapy they were not significantly different from baseline values. Fluctuations in the plasma concentrations of phenytoin between doses were relatively small and it is unlikely that any small variations in blood sampling times or times of drug administration could be responsible for the observed fall in plasma phenytoin concentration on week five of vigabatrin.

Antipyrine tests

The antipyrine kinetic parameters in seven of the eight patients before and during vigabatrin therapy are shown in Table 2. There was no consistent change in antipyrine clearance or half-life after 4 weeks of vigabatrin. Unfortunately, patient 5 failed to complete successfully his second antipyrine test so that there was paired data for only seven subjects.

Plasma protein binding

Values for the plasma phenytoin free fraction are shown in Table 3. The free fraction in the three patients taking sodium valproate was markedly higher than in the other five patients, which is in keeping with the known interaction between phenytoin and valproate (Dahlqvist *et al.*, 1979). There was quite marked variation between the phenytoin binding measured on the two occasions in some of the patients but there was no consistent change during vigabatrin therapy.

In vitro *binding*

The mean free fraction of phenytoin was 0.139 ± 0.00425 (± s.d.) ($n = 5$) and when vigabatrin was added to the plasma as well the free fraction was 0.137 ± 0.00382 (± s.d.) ($n = 5$). Thus the protein binding of phenytoin was not altered by addition of vigabatrin *in vitro*.

Table 2 Antipyrine half-life and clearance before and during vigabatrin therapy

	Baseline			*During vigabatrin*		
Patient	$t_{1/2}$ *(h)*	*AUC* *(μg ml^{-1} h)*	*Clearance* *(ml min^{-1})*	$t_{1/2}$ *(h)*	*AUC* *(μg ml^{-1} h)*	*Clearance* *(ml min^{-1})*
01	5.99	110.97	90.10	6.09	80.80	123.75
02	6.22	139.16	71.85	6.16	148.96	67.13
03	6.12	106.04	94.30	6.34	93.86	106.53
04	3.49	56.80	176.03	3.78	79.35	126.02
06	4.56	157.05	63.67	7.41	260.41	38.40
07	10.39	176.06	56.79	8.24	107.90	92.67
08	7.08	66.73	149.84	7.05	89.39	111.86
Mean	6.27	116.12	100.37	6.44	122.96	95.20
± s.d.	±2.17	±45.00	±45.40	±1.40	±65.12	±32.12

$t_{1/2}$ = elimination half-life of antipyrine, AUC = area under the curve.

Table 3 Protein binding of phenytoin

	Phenytoin free fraction	
Patient	*Baseline*	*Vigabatrin*
01	0.146	0.106
02	0.111	0.143
03*	0.217	0.211
04*	0.214	0.152
05	0.163	0.137
06	0.100	0.139
07*	0.197	0.188
08	0.121	0.134
Mean	0.159	0.151
± s.d.	0.047	0.033

Table 4 Urinary *p*-HPPH:DPH ratios

Patient number	*Control period*	*Vigabatrin therapy*
01	30.2	65.9
02	23.5	22.5
03	65.6	53.6
04	78.0	87.4
05	82.0	41.3
06	139.5	129.5
07	53.5	135.8
08	28.2	44.6
Mean	62.6	72.1
± s.d.	±38.6	±41.6

* patients on sodium valproate.

Urinary p-*HPPH:DPH ratios*

Table 4 shows the urinary *p*-HPPH:DPH ratio for each patient before starting vigabatrin and at the end of 4 weeks' full dose vigabatrin therapy. All patients had fairly high ratios when measured in the control period which is consistent with their state of enzyme induction. However, there was no consistent change in the ratio when measured the second time during the vigabatrin treatment period. In fact, there appeared to be a marked degree of random variation in the ratios, which may or may not represent true biological variation in the amount of phenytoin and *p*-HPPH excretion. Because the *p*-HPPH and phenytoin excretion was expressed as a ratio, any incompleteness in the 24 h urine collections should be unimportant, provided the rate of excretion of the two compounds is constant throughout 24 h. Although the absolute amounts of phenytoin excreted unchanged in the urine were low, they were well within the assay limits of detection, so inaccuracies in the phenytoin measurements should not have been the cause of the apparently random fluctuations in the ratio.

Compliance

Compliance, as assessed by medication record charts, was excellent.

Discussion

The interaction between vigabatrin and phenytoin has been confirmed. The mean decrease in plasma phenytoin concentration was 23%, which is a relatively small change but probably of clinical significance. In view of the fairly small fall in plasma phenytoin concentration that we have observed, it would be reassuring to see the result confirmed in a larger study. The time course of the interaction is surprising. The fall in plasma phenytoin concentration only became apparent after 4 weeks of full dose (3 g daily) vigabatrin treatment. This unusual time course cannot be explained by a systematic error in the phenytoin assay on that particular week as all the plasma samples were assayed together at the end of the study. A possible explanation is that the delay is due to an enzyme induction process which required several weeks to reach maximal effect, although a sudden rather than a gradual fall in phenytoin concentration by this mechanism would be unusual. However, the antipyrine tests did not show any evidence of a consistent change in the state of induction of hepatic drug metabolism. This group of patients was already enzyme-induced compared with reported control values from normal volunteers, with mean antipyrine half-life of 6.27 h and clearance of 100.37 ml min^{-1} (Perucca *et al.*, 1984). It was not possible to demonstrate a further shortening of antipyrine half-life or an increase in antipyrine clearance during vigabatrin therapy. This may have been because the antipyrine test is too insensitive to determine any relatively subtle changes in liver metabolic capacity in subjects who are already enzyme-induced. In addition there was no consistent change in the urinary *p*-HPPH:DPH ratios when the patients were taking vigabatrin. There is no evidence, therefore, to suggest an enzyme-induction mechanism as the cause of the observed interaction between vigabatrin and phenytoin. Vigabatrin is excreted largely unchanged in the urine, with relatively little being metabolised in the liver. It would be an unusual finding for a drug which itself is little metabolised by the liver to have a significant effect on hepatic enzymes. It would possibly have been more sensitive to use a tracer dose of [^{14}C]-phenytoin to measure phenytoin half-life (Houghton & Richens, 1974b) before and during vigabatrin therapy although it was felt that the use of radiolabelled phenytoin in patients could not be justified in this preliminary investigation. The study did not show any change in the plasma protein binding of phenytoin during vigabatrin therapy. Browne *et al.* (1987) also comment that no change in binding occurs with addition of vigabatrin. If displacement from protein binding sites had been the mechanism of interaction one would have expected an almost immediate reduction in total plasma phenytoin levels rather than the delayed fall which we observed. *In vitro* studies also showed no binding interaction.

In conclusion, although this study has confirmed that an interaction between vigabatrin and phenytoin probably does occur, resulting in a reduction in total plasma phenytoin concentrations, it appears to be a delayed effect and it has not been possible to demonstrate the mechanism by which it occurs. Further studies using larger numbers of subjects are required to elucidate this and should include measurement of phenytoin half-life before and during vigabatrin therapy. If the interaction is not due to displacement of phenytoin from plasma proteins or to induction of phenytoin metabolism, another possibility is an increase in tissue binding sites for phenytoin resulting in an increased volume of distribution.

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