Unbound phenytoin plasma concentrations in patients comedicated with sodium valproate—the predictive value of plasma albumin concentration

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1 Phenytoin protein binding in epileptic patients on phenytoin as monotherapy has been compared with protein binding in patients treated with both phenytoin and sodium valproate. In addition the relative value of assayed total phenytoin plasma concentrations and assayed unbound phenytoin plasma concentrations and the value of predicted unbound phenytoin plasma concentrations in predicting phenytoin toxicity has been assessed.

2 The mean phenytoin unbound fraction for patients taking sodium valproate (0.122) was significantly greater than for those on monotherapy (0.082).

3 There were six episodes of clinical toxicity. In five toxic episodes the assayed unbound phenytoin plasma concentration was a better reflection of toxicity than the assayed total phenytoin plasma concentration, and four of these occurred in patients on sodium valproate.

4 Unbound phenytoin plasma concentrations were predicted from a single regression equation correlating all assayed total phenytoin plasma concentrations with assayed unbound phenytoin plasma concentrations, from two separate regression equations for each group of patients, and from the correlation between phenytoin protein binding and plasma albumin concentration.

5 The unbound phenytoin plasma concentrations predicted from the two regression equations were statistically no less effective than the assayed unbound phenytoin plasma concentrations in assessing toxicity.

6 Despite a correlation between plasma albumin concentrations and phenytoin protein binding, the use of albumin concentrations in predicting unbound phenytoin plasma concentrations appeared to be of little additional benefit.

Keywords valproate phenytoin protein binding plasma proteins epilepsy

Introduction

Monitoring of the total phenytoin plasma concentration (TPPC) is a well accepted guide to the effective clinical use of this drug. In otherwise healthy epileptic patients not receiving drugs known to displace phenytoin from plasma protein binding sites, nor with concurrent medical

conditions associated with reduced protein binding, the routinely assayed TPPC correlates well with both anticonvulsant effect and toxicity (Kutt *et al.*, 1964; Kutt & McDowell, 1968; Lund, 1974).

Phenytoin is highly bound to plasma proteins.

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Monitoring therapy using TPPC assumes there is minimal interindividual variability in phenytoin protein binding and that the TPPC accurately reflects the unbound phenytoin plasma concentration (UPPC), presumed to be the pharmacologically active component. It is well recognized that phenytoin protein binding is reduced in the presence of sodium valproate, sodium valproate displacing phenytoin from protein binding sites (Perucca *et al.*, 1980; Tsanaclis *et al.*, 1984). In addition, sodium valproate has been shown to inhibit phenytoin metabolism (Perucca *et al.*, 1980). Thus in the presence of sodium valproate, it may be that the assay of TPPC could be misleading, and monitoring may be improved by the direct estimation of UPPC.

In addition to the reduction in phenytoin protein binding produced by sodium valproate, phenytoin binding is also reduced in patients with low plasma albumin concentrations (Hooper *et al.*, 1974). A correlation between plasma albumin concentration and the percentage of unbound phenytoin has previously been reported (Porter & Layzer, 1975; Kilpatrick *et al.*, 1984). Thus, plasma albumin concentration is a determinant of phenytoin protein binding and may be useful in predicting UPPC.

The aims of this study were threefold; firstly, to compare the range of phenytoin protein binding in a group of epileptic patients treated with phenytoin alone, and a group treated with both phenytoin and sodium valproate; secondly, to assess the relative value of the assay of unbound and total plasma concentrations of phenytoin in the two groups of patients; thirdly, since the assay of UPPC is both time consuming and expensive, to assess the value of different predictions of UPPC as compared to assayed UPPC.

Methods

Patients

Sixty-three plasma samples were obtained from 46 patients. More than one plasma sample was taken from 11 patients in whom there was a dose change. The subjects were consecutive patients treated with phenytoin attending the Anticonvulsant Clinic, The Royal Melbourne Hospital. Twenty-eight patients were treated with both phenytoin and sodium valproate and 18 patients with phenytoin alone. There were 25 females and 21 males; ages ranged from 19 to 76 years. No patient was pregnant or had renal or hepatic dysfunction as assessed by the usual clinical and laboratory indices, and in all but one patient the plasma albumin concentration was within the

normal reference range ($35\text{--}50\text{ g l}^{-1}$). Patients were prescribed no other drugs known to alter phenytoin protein binding apart from sodium valproate. All patients were assessed for symptoms and signs of phenytoin toxicity according to conventional clinical criteria (nystagmus, ataxia, drowsiness, dysarthria, diplopia) in the absence of knowledge of the phenytoin plasma concentrations. A clinical diagnosis of phenytoin toxicity was made if a patient exhibited two or more of the above symptoms or signs.

Sample collection

Samples (10 ml) of venous blood were collected in the outpatient clinic and plasma was obtained by centrifugation. Plasma samples were then subjected to ultrafiltration using a micropartition filter (Centrifree, Amicon Corp.) and centrifugation at 2000 *g* in a fixed angle centrifuge for 30 min at a constant temperature of $20\text{--}22^\circ\text{C}$ using a previously published method (Kilpatrick *et al.*, 1984). An ultrafiltrate volume of at least 200 μl was obtained. Ultrafiltrate and plasma samples were stored at -20°C . Prior to centrifugation and ultrafiltration the pH of plasma specimens was in the range 7.4–7.8 (Kilpatrick *et al.*, 1984).

Analytical methods

Phenytoin in plasma (total) and ultrafiltrate (unbound) was assayed using liquid chromatography.

Plasma specimens (0.5 ml) containing 25 μg butabarbitalone as internal standard were acidified with 250 μl hydrochloric acid and extracted with 5 ml dichloromethane for 2 min using a vortex mixer. After centrifugation the aqueous phase was removed and the organic phase was evaporated to dryness at 35°C under a gentle stream of nitrogen. The extract was reconstituted with 50 μl of mobile phase of which 3 μl was injected into the chromatograph, a Hewlett Packard 1090A liquid chromatograph equipped with a diode array detector. Assays were performed using a $100 \times 2.1\text{ mm}$ Hypersil ODS column (Hewlett Packard) at 50°C . The mobile phase was methanol : 0.01 mol l^{-1} phosphate buffer pH 3.0 (42 : 58) delivered at 0.5 ml min^{-1} . Ultraviolet absorbance was monitored at 224 nm. Peak heights were quantitated using a Hewlett Packard 3392A integrator.

Ultrafiltrates were injected directly into the liquid chromatograph using the same operating conditions. Assay precision for unbound phenytoin in the ultrafiltrates was assessed by processing replicate plasma specimens containing

total phenytoin concentrations of $16 \mu\text{mol l}^{-1}$ and $60 \mu\text{mol l}^{-1}$. The coefficients of variation were 2.6% ($n = 6$) and 3.4% ($n = 6$) respectively which are comparable with those previously reported (Kilpatrick *et al.*, 1984). Binding of phenytoin to the ultrafiltration membrane was negligible (Kilpatrick *et al.*, 1984).

Plasma albumin concentrations were determined using the standard Technicon SMAC system albumin (BCG) method (Technicon method number SG4-0030FM9, 1979); normal range: 35–50 g l^{-1} .

Data analysis

Association between variables was obtained by linear regression analysis and calculation of correlation coefficients. The 90% predictability limits for the prediction of the UPPC from a single assayed TPPC were calculated according to the method of Armitage (1971). The unbound fraction of phenytoin was calculated by dividing the assayed UPPC by the assayed TPPC, and expressed as a percentage.

Three predictions for the unbound concentration of phenytoin were calculated from the assayed TPPC:

1. Unbound phenytoin concentration predicted from the single regression equation obtained by the linear regression of the assayed UPPC against assayed TPPC data for all patients (Figure 1).
2. Unbound phenytoin concentration predicted from the two regression equations obtained by the linear regression of the assayed UPPC against assayed TPPC data for patients on monotherapy and for patients on concurrent sodium valproate therapy (Figure 2).
3. Unbound phenytoin concentration predicted from the regression equations obtained by linear regression of the unbound fraction of phenytoin (assayed) against the plasma albumin concentration for patients on phenytoin alone and for patients also on sodium valproate (Figure 3). The unbound fraction was predicted from the assayed plasma albumin concentration and multiplied by the assayed TPPC to predict the UPPC.

Four data sets, each containing 63 records, were then constructed. In the first data set the patient identification code, the assayed TPPC, the assayed UPPC, and symptoms and signs of phenytoin toxicity were listed. In the second data set UPPCs predicted from the single regression equation replaced the assayed UPPCs

while in the third data set UPPCs predicted from the two regression equations replaced the assayed UPPCs. In the fourth data set UPPCs predicted from plasma albumin concentrations replaced the assayed UPPC.

In each data set, individual records were listed in order of increasing UPPCs. For each data set, the proportion of highest UPPCs needed to include all cases of clinical toxicity was recorded. These proportions were compared between tables by chi square analysis.

Results

Phenytoin unbound fraction

The phenytoin unbound fraction for all patients ranged from 4.8% to 19.2% with a mean of 10.6%. The fraction was significantly different for patients receiving monotherapy (range 4.8%–16.3%) as compared to those receiving concurrent sodium valproate (range 6.6%–19.2%) therapy ($t = 4.79$, $\text{df} = 61$, $P < .001$, unpaired t -test). The mean phenytoin unbound fraction for patients on monotherapy was 8.2% (s.d. 2.6) and for patients receiving concurrent sodium valproate therapy was 12.2% (s.d. 2.9).

Relationship between total and unbound phenytoin plasma concentrations

Overall there was a significant linear correlation between assayed total and assayed unbound phenytoin plasma concentrations ($y = 0.082x + 0.967$; $r = 0.72$; $P < 0.001$) (Figure 1). Of the five values above the 90% predictability limit, all patients were receiving concurrent sodium valproate therapy. One patient whose UPPC was below the 90% predictability limit, was receiving phenytoin alone.

There was a stronger correlation between assayed TPPCs and assayed UPPCs for patients on monotherapy ($y = 0.062x + 0.778$; $r = 0.84$; $P < 0.001$) (Figure 2) and for patients on both drugs ($y = 0.107x + 0.608$; $r = 0.83$; $P < 0.001$) (Figure 2) than for the group as a whole (see above), confirming a significant difference in the protein binding of each group of patients.

Phenytoin toxicity

There were six episodes of phenytoin toxicity. In one patient both TPPCs and UPPCs accurately reflected the toxic state. This patient was not receiving concurrent sodium valproate therapy. In the remaining five patients, the assayed UPPC was a better reflection of toxicity than was

Figure 1 Correlation of assayed total and unbound phenytoin plasma concentrations. Solid squares ($\blacksquare$) represent cases where the assayed UPPC falls outside the 90% predictability limits for the prediction of the UPPC from an assayed TPPC (---).

Figure 2 Correlation of assayed total and unbound phenytoin plasma concentrations for patients receiving sodium valproate ($\square$) and patients receiving phenytoin alone ($\blacksquare$).

the TPPC. Four of these five toxic episodes occurred in patients taking sodium valproate and all had significantly reduced protein binding such that the UPPCs fell on or above the 90% predictability limits (Figure 1).

All patients with clinical features of phenytoin toxicity had UPPCs greater than $7.0 \mu\text{mol l}^{-1}$, whereas all had TPPCs below $71 \mu\text{mol l}^{-1}$ and in three cases the TPPC was less than $60 \mu\text{mol l}^{-1}$ (therapeutic range $40\text{--}80 \mu\text{mol l}^{-1}$).

Relationship between phenytoin unbound fraction and plasma albumin concentration

There was a negative correlation between plasma albumin concentration (within the normal reference range) and the phenytoin unbound fraction in patients on monotherapy ($y = -0.43x + 27.04$; $r = 0.50$, $P < 0.01$, $df = 35$) (Figure 3). There was a similar correlation between plasma albumin concentration and the phenytoin unbound fraction in patients on both phenytoin and sodium valproate ($y = -0.43x + 30.89$; $r = 0.50$, $P < 0.01$, $df = 24$) (Figure 3).

Relationship between phenytoin unbound fraction and total valproate plasma concentrations

There was a weak correlation between total valproate concentration x , and the phenytoin

unbound fraction y , ($y = 0.006x + 9.89$; $r = 0.43$, $P < 0.01$, $df = 35$).

Correlation between predicted unbound phenytoin concentrations and assayed unbound phenytoin concentrations

The correlations between assayed UPPCs, and UPPCs predicted from the two regression equations ($r = 0.85$; $df = 61$, $P < 0.001$) and UPPCs predicted from albumin concentrations alone ($r = 0.90$; $df = 61$, $P < 0.001$) were stronger than the correlation between assayed UPPCs and UPPCs predicted from the single regression equation ($r = 0.72$; $df = 61$, $P < 0.001$).

Correlation between clinical toxicity and assayed unbound phenytoin concentrations, and clinical toxicity and predicted unbound phenytoin concentrations

The 33 highest total phenytoin plasma levels encompassed the six clinically toxic patients in this study; however, only the 15 highest assayed UPPCs needed to be encompassed to include these patients. When the UPPCs predicted from the single regression equation were used, the 33 highest UPPCs were needed to encompass all toxic patients. When the UPPCs predicted from the two regression equations were used, the 22

Figure 3 Correlation of plasma albumin concentration and phenytoin unbound fraction for patients receiving sodium valproate ($\square$) and patients receiving phenytoin alone ($\blacksquare$). Data from one patient receiving phenytoin and sodium valproate who had a plasma albumin level of 54 g l^{-1} and a phenytoin unbound fraction of 6.1% , are not shown.

highest UPPCs in each data set were needed to encompass all toxic patients. When the UPPCs predicted using albumin concentrations were used, the 21 highest UPPCs were needed to encompass all toxic patients.

There was a significant difference between the proportion of UPPCs that included all toxic patients when the values predicted from the single regression equation were compared with the assayed values ($\chi^2 = 10.90$, $P < 0.001$). There was no significant difference between the proportion of UPPCs predicted from the two regression equations ($\chi^2 = 1.87$, $P > 0.10$), or those predicted from albumin concentrations ($\chi^2 = 1.40$, $P > 0.20$) when compared with the proportion of assayed UPPCs needed to include all toxic patients.

The difference between the proportion of UPPCs predicted from the two regression equations ($\chi^2 = 3.90$, $P < 0.05$), or from albumin concentrations ($\chi^2 = 4.67$, $P < 0.05$) and the proportion of UPPCs predicted from the single regression line needed to include all toxic patients was statistically significant.

If assayed values of UPPCs greater than or equal to $7 \mu\text{mol l}^{-1}$ are accepted as indicative of toxicity, then four episodes of clinical toxicity would have been misclassified using the single regression line method, three using the two regression lines method, and two using the albumin method.

Discussion

This study of 46 patients treated with phenytoin, including 28 patients concurrently treated with sodium valproate, has demonstrated a four fold range of phenytoin protein binding. Importantly, the mean phenytoin unbound fraction was significantly higher in patients on both drugs compared with those on monotherapy. Thus if UPPCs are to be estimated from TPPCs, the effect of concurrent sodium valproate therapy must be taken into consideration.

Although a number of studies have investigated the phenytoin-sodium valproate interaction and have advocated that therapy in these patients would be better monitored with UPPCs (Knott *et al.*, 1982; Perucca, 1984; Cramer *et al.*, 1984; Levy & Moreland, 1984), few studies have assessed clinically the value of UPPCs. Several have shown that phenytoin saliva concentrations, which reflect unbound plasma concentrations, correlate with the clinical efficacy of the drug (McAuliffe *et al.*, 1977; Knott *et al.*, 1982; Reynolds *et al.*, 1982). Two previous studies have reported a better correlation between phenytoin toxicity and UPPCs than with TPPCs

(Booker & Darcey, 1973; DeMonaco & Lawless, 1983), but this relationship has not been studied in a large group of patients treated concurrently with sodium valproate.

As there is considerable interindividual variation in each case of epilepsy in terms of both the nature and frequency of seizures it is difficult to correlate UPPCs with clinical efficacy across a patient population. This study therefore used a clinical diagnosis of phenytoin toxicity to assess the relative usefulness of both assayed UPPCs and TPPCs, and predicted UPPCs.

The present study has found that in most cases of phenytoin toxicity the clinical state correlated better with the UPPC than the TPPC and in no case was the correlation with the TPPC better than with the UPPC. Of the five episodes where UPPCs correlated better than TPPCs with toxicity, four patients were concurrently treated with sodium valproate.

These findings suggest that in patients treated with both phenytoin and sodium valproate, the TPPC may be misleading and patients would be better monitored with UPPCs. As this additional measurement is both time consuming and expensive, the value of using predicted UPPCs was assessed.

If the effect of sodium valproate on phenytoin protein binding is not considered and the single regression equation obtained from all assayed UPPCs and assayed TPPCs is used to predict the UPPC, then clearly the assayed UPPCs correlate better with toxicity than do the predicted UPPCs.

If the effect of sodium valproate on phenytoin binding is considered and two separate regression equations are derived from the assayed UPPC and TPCC data, one for each group of patients, then the predicted UPPCs are statistically no less accurate than the assayed UPPCs when correlated with phenytoin toxicity. Despite a correlation between plasma albumin concentration and phenytoin protein binding, the use of the albumin concentration does not improve the correlation between predicted UPPCs and phenytoin toxicity. However, in disease states in which plasma albumin levels are low, the findings may well be different.

We conclude that assessment of the UPPC is of importance in monitoring the therapy of patients treated with both phenytoin and sodium valproate. This can be done by direct measurement, but as an alternative to the direct measurement of UPPCs, UPPC and TPPC data can be obtained for both groups of patients and used to derive regression equations from which UPPCs can then be predicted. The use of plasma albumin concentrations in predicting UPPCs

appears to be of little additional benefit in patients whose albumin is within the normal range.

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