The prediction of steady-state plasma phenobarbitone concentrations (following low-dose phenobarbitone) to refine its use as an indicator of compliance

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- 1 A model for predicting the steady-state plasma concentration of phenobarbitone following low-dose phenobarbitone used as an indicator of compliance was derived using data for 10 healthy volunteers.
- 2 Each volunteer was given a single 30 mg oral dose of phenobarbitone and the pharmacokinetics were described. Subsequently, volunteers were given phenobarbitone 2 mg daily for 28 days and a further pharmacokinetic profile determined during and after this period.
- 3 An initial predicted estimate of steady-state plasma drug concentration was made using each volunteer's demographic details. This estimate was revised by Bayesian analysis using single timed samples (24, 48, 72 or 96 h) following the single dose.
- 4 The model was tested on a further 10 healthy volunteers given a single 8 mg dose and who were subsequently given 2 mg daily for 28 days.
- 5 The revised estimate of peak steady-state plasma phenobarbitone concentration utilising the 96 h post-single dose concentration (356 ng ml^{-1}) was least biased (mean prediction error $\pm 95\% \text{ CI} = 10.6 \pm 19.8 \text{ ng ml}^{-1}$) and most precise (root mean square error $\pm 95\% \text{ CI} = 28.3 \pm 19.0 \text{ ng ml}^{-1}$). In all cases the peak or trough steady-state drug concentration was within 13% of the predicted value.
- 6 The model reflected compliance accurately in a further eight volunteers with simulated partial (two-thirds) compliance.
- 7 The use of a predictive model using Bayesian analysis to estimate expected steady-state plasma phenobarbitone concentrations could increase further the usefulness of low-dose phenobarbitone as an indicator of compliance.

Keywords compliance phenobarbitone

Introduction

We have recently developed the use of low-dose phenobarbitone as a pharmacological indicator of compliance (Feely *et al.*, 1987). Subsequently we have used it to assess compliance in a number of clinical situations (Bignell *et al.*, 1988; Kumar *et al.*, 1989a; Penn *et al.*, 1988; Pullar *et al.*, 1988a,c) and have shown it to be more effective than traditional methods (Pullar *et al.*, 1988a, 1989). Low-dose phenobarbitone is a suitable pharmacological indicator for a number of reasons. The drug has a long elimination half-life which means that it gives information on compliance over the preceding few weeks, rather than days, which cannot be manipulated by merely taking one or two standard doses immediately before a clinic visit (the so-called 'tooth brush' effect).

At the doses used (around 2 mg daily) it has no measurable enzyme-inducing (Price *et al.*, 1986) or toxic (Kumar *et al.*, 1989b; Pullar *et al.*, 1988a) effects. Once correction is made for age and body weight there is relatively little interindividual and even less intraindividual variation in pharmacokinetics. However, the highest recorded steady-state plasma drug concentration in age-related healthy volunteers was 50% higher than the lowest (Feely *et al.*, 1987). This means that an individual with poor compliance who, if compliance had been complete, would fall in to the upper part of the range, might be misclassified as having good compliance. On the other hand, because of the difficulty in ensuring 100% compliance over several weeks even in well motivated

healthy volunteers, it is possible that the lower end of the volunteer range is artefactually low. In an attempt to overcome these problems of defining a 'normal range' and to increase the accuracy of the technique, we have developed a model to predict the (very low) steady-state plasma phenobarbitone concentrations associated with full compliance.

Methods

Approval was obtained from the Leeds West Research Ethics Committee and written consent was obtained from all volunteers. Three separate studies were carried out.

Study 1

Ten healthy volunteers (seven male, three female), mean ($\pm$ s.d.) age 29 (± 4) years were studied. The subjects were taking no medicines known to affect the hepatic metabolism of phenobarbitone. Mean ($\pm$ s.d.) alcohol consumption was 9.8 ± 8 units per week. All subjects maintained their usual alcohol consumption. Nine were non-smokers and one smoked 5–6 cigarettes daily. Following an overnight fast, 30 mg phenobarbitone (CP Pharmaceuticals) was given as a single oral dose with 250 ml water. No food or drink was allowed for the following 2 h. Venous blood (5 ml) was taken before dosing and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 168, 216, 264, 336 and 504 h post-dose. Repeated dosing with phenobarbitone 2 mg daily for 28 days was commenced 4 weeks after the last blood sample. During chronic dosing phenobarbitone was taken between 08.30 h and 09.00 h each day. Ingestion was supervised directly on weekdays and at weekends volunteers were telephoned with a reminder to take their tablets. The 2 mg phenobarbitone tablets were prepared by the School of Pharmacy, University of Manchester. On days 4, 7, 14 and 21 venous blood was taken immediately pre-dose and at 6 h post-dose. On day 28 venous blood was taken pre-dose and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 168 and 240 h post dose.

All blood samples were drawn into tubes containing lithium heparin. Plasma was stored at -20°C and phenobarbitone concentration was subsequently analysed by reversed phase h.p.l.c. (Peaker *et al.*, 1989). The lower limit of assay (signal to noise ratio = 3) was 50 ng ml^{-1} . The inter- and intra-assay coefficients of variation at concentrations between 50 ng ml^{-1} and 500 ng ml^{-1} were 4.8% and 3.3%, respectively. The peak plasma phenobarbitone concentration (C_{max}) and the time to peak concentration (t_{max}) following the single dose and the last of the repeated doses were noted. The area under the plasma drug concentration-time curve was calculated for the single dose ($\text{AUC}(0,\infty)$) and at steady-state ($\text{AUC}(0,24)$) using the linear trapezoidal rule. Clearance (CL) was derived from $\text{Dose} \times F/\text{AUC}$ where bioavailability (F) was assumed to be 1 (Viswanathan *et al.*, 1978; Wilensky *et al.*, 1982). The elimination rate constant (k) was calculated by linear least squares regression analysis of the terminal slope of the log plasma drug concentration-time curve. The elimination half life ($t_{1/2,z}$) was derived from $0.693/k$. All pharmacokinetic variables were corrected, where

appropriate, for weight. The apparent volume of distribution (V) was derived from CL/k . The means of these pharmacokinetic parameters will be referred to as the initial estimates.

Statistical analysis of differences between the pharmacokinetic parameters following the single dose and at steady-state was carried out using a paired *t*-test. Using the initial estimates of pharmacokinetic parameters for each subject predictions were made of the peak and trough phenobarbitone concentrations during chronic dosing. Standard equations for a one compartmental model with first order elimination and absorption were used. From the individual plasma phenobarbitone concentration-time profiles after a single dose the peak concentrations were maintained from 1–10 h post-dose and therefore peak predictions were made when $t = 6$ h post dose.

The initial estimates of pharmacokinetic parameters were refined by a Bayesian analysis (Sheiner & Beal, 1982) software package (Chrystyn *et al.*, 1988) into revised estimates of pharmacokinetic parameters in the light of the phenobarbitone concentration 24 h after the single dose (24 Bayesian). Similar revised estimates were obtained using plasma phenobarbitone concentrations 48 h (48 Bayesian), 72 h (72 Bayesian) and 96 h (96 Bayesian) following the single dose. Each set of revised estimates was used to predict the steady-state peak and trough plasma phenobarbitone concentrations on chronic dosing with 2 mg day^{-1} . The performance in terms of the mean prediction error (MPE) and root mean square error (RMSE) were calculated for each version of the predictive model (Sheiner & Beal, 1981). The relative performance was evaluated to determine any statistically significant difference between each prediction (Sheiner & Beal, 1981).

Study 2

In order to test the predictive model devised from Study 1, 10 further healthy volunteers (seven male and three female) were studied. Their mean ($\pm$ s.d.) age was 31 (± 6 years). All were non-smokers and the mean ($\pm$ s.d.) alcohol consumption was $11 (\pm 8)$ units per week. After an overnight fast the volunteers were given 8 mg phenobarbitone orally (Pharmacy Department, University of Manchester). Venous blood was taken 24, 48, 72 and 96 h post dose. After at least 4 weeks volunteers were commenced on phenobarbitone 2 mg daily for 28 days with timing and supervision as in study 1. Venous blood was taken pre-dose and 6 h post-dose on day 28. Phenobarbitone was assayed as in Study 1.

Using the initial estimates of pharmacokinetic parameters derived from study 1 estimates were made for each volunteer in Study 2. Revised estimates for each individual's pharmacokinetic parameters were made using Bayesian analysis and their 24, 48, 72 and 96 h concentrations post-dose. For each individual's set of kinetic parameters (initial estimates 24, 48, 72 and 96 Bayesian respectively), predictions of steady state plasma phenobarbitone concentration of peak ($t = 6$ h) and trough ($t = 24$ h) were made as in Study 1. The mean prediction error (MPE) and root mean square error (RMSE) were calculated for each version of the predictive model as in Study 1.

Study 3

Eight healthy volunteers (six males, two females) with a mean ($\pm$ s.d.) age of 32 ($\pm$ 6) years were studied. Volunteers were given a single 30 mg ($n = 4$) or 8 mg ($n = 4$) oral dose of phenobarbitone as described above and venous blood was taken at 96 h. At least 4 weeks later the volunteers were started on phenobarbitone 2 mg for 28 days but missed their dose on day 2 and on every third day thereafter with supervision as described above. Venous blood was taken pre-dose and 6 h post-dose on day 28. Predictions of expected steady-state plasma drug concentrations were made from initial estimates and the Bayesian derived revised estimates using the 96 h post-single dose phenobarbitone concentration. Seven of these volunteers were also dosed on a separate occasion with phenobarbitone 2 mg daily for 28 days and pre-dose 2 h and 6 h post-dose samples taken on day 28. To enable comparison with the expected ranges (Feely *et al.*, 1987) phenobarbitone concentrations were expressed as a ratio of dose (LDR) where $LDR =$

$$\frac{\text{plasma concentration (mg l}^{-1}\text{)} \times \text{body weight (kg)}}{\text{dose (mg)}}$$

The plasma concentrations used to derive the LDRs were a) the measured concentration following simulated poor (two-thirds) compliance b) the measured concentration following full compliance, c) the initial assuming full compliance and d) the revised 96 h Bayesian estimate assuming full compliance.

Results

The plasma phenobarbitone concentrations-time curves following the single dose and at steady-state for the 10 volunteers in Study 1 are shown in Figure 1. The $C_{\max}$ after the single dose was 893 ± 140 ng ml $^{-1}$ (mean $\pm$ s.d.) with a $t_{\max}$ of 4.1 ± 3.1 h. At steady-state the mean trough concentration was 308 ± 46 ng ml $^{-1}$ and the mean peak concentration was 384 ± 60 ng ml $^{-1}$. The peak steady-state concentration was reached after a

Figure 1 Plasma phenobarbitone (PB) concentrations (mean $\pm$ s.d.; $n = 10$) vs time after a single oral dose of 30 mg PB (—) and at steady state (----) after repeated daily doses of 2 mg PB for 28 days.

mean of 1.4 ± 0.5 h after dosing. This level was then maintained for at least a further 8 h in all subjects and the mean intra-individual variation over this period was $7.3 \pm 1.9\%$.

The values of CL, $t_{1/2,z}$ and V following single and repeated dosing for each of the ten volunteers in Study 1 are shown in Table 1. Using a paired t -test there was no significant difference between these parameters following the single dose when compared with the values at steady-state. Table 2 shows the mean prediction error and root mean square error for predicted peak and trough steady-state concentrations using each of the post single dose phenobarbitone concentrations separately. Relative performance showed that the differences were not significant.

The mean prediction error and root mean square errors for the predictions in Study 2 are shown in Table 3. Again, relative performance revealed no statistically significant difference.

Figure 2 shows the actual plasma drug concentrations to dose ratios on day 28 for the eight subjects with simulated two-thirds compliance along with the predicted steady-state concentrations based on the initial estimate, those based on the 96 h post-single dose sample assuming complete compliance and also the measured steady-state concentrations previously obtained for seven of the volunteers following daily dosing for 28 days.

Table 1 Values of clearance (CL), elimination half life ($t_{1/2,z}$) and apparent volume of distribution (V) for each of ten volunteers following a single dose of phenobarbitone 30 mg and at steady state after repeated daily doses of phenobarbitone 2 mg for 28 days

Subject	Single dose			Steady state		
	CL (ml h $^{-1}$ kg $^{-1}$)	$t_{1/2,z}$ (h)	V (l kg $^{-1}$)	CL (ml h $^{-1}$ kg $^{-1}$)	$t_{1/2,z}$ (h)	V (l kg $^{-1}$)
1	3.17	105	0.47	3.27	138	0.66
2	5.10	74	0.54	4.83	52	0.36
3	2.81	132	0.53	2.87	127	0.52
4	4.21	95	0.57	4.35	105	0.65
5	2.31	138	0.46	2.57	145	0.53
6	4.26	95	0.58	4.23	92	0.56
7	2.86	117	0.48	3.06	138	0.60
8	3.86	105	0.58	3.70	113	0.60
9	2.76	122	0.50	2.77	127	0.48
10	3.07	117	0.51	3.31	105	0.46
Mean	3.44	110	0.52	3.50	114	0.54
s.d.	0.87	19.2	0.05	0.76	27.6	0.09

Table 2 Mean measured and predicted trough ($t = 0$ h) and peak ($t = 6$ h) plasma phenobarbitone concentration at steady-state in Study 1 with the mean prediction error (bias) and root mean square error (precision) $\pm$ 95% confidence intervals for the initial estimate and the Bayesian analysis

Time	Mean concentration (ng ml ⁻¹) ($\pm$ s.d.)		Mean prediction error (ng ml ⁻¹) ($\pm$ 95% confidence interval)		Root mean square error (ng ml ⁻¹) ($\pm$ 95% confidence intervals)	
	0 h	6 h	0 h	6 h	0 h	6 h
Measured	312 (49)	367 (54)				
Initial estimate	299 (36)	332 (40)	-13.7 (41.7)	-34.9 (45.4)	56.9 (43.4)	69.6 (46.1)
Bayesian 24 h	305 (44)	338 (48)	-7.2 (34.9)	-28.3 (36.2)	46.9 (35.2)	55.6 (37.7)
Bayesian 48 h	325 (58)	360 (63)	12.7 (30.7)	-6.7 (31.5)	42.7 (32.2)	42.3 (32.7)
Bayesian 72 h	322 (63)	356 (65)	10.0 (23.6)	-10.2 (21.2)	32.9 (25.2)	29.9 (23.0)
Bayesian 96 h	326 (63)	361 (64)	14.0 (18.4)	-5.9 (17.5)	28.1 (29.4)	23.9 (16.9)

Table 3 Mean measured and predicted trough ($t = 0$ h) and peak ($t = 6$ h) plasma phenobarbitone concentration at steady-state in Study 2 with the mean prediction error (bias) and root mean square error (precision) $\pm$ 95% confidence intervals for the initial estimate and the Bayesian analysis

Time	Mean concentration (ng ml ⁻¹) ($\pm$ s.d.)		Mean prediction error (ng ml ⁻¹) ($\pm$ 95% confidence interval)		Root mean square error (ng ml ⁻¹) ($\pm$ 95% confidence intervals)	
	0 h	6 h	0 h	6 h	0 h	6 h
Measured	317 (44)	345 (54)				
Initial estimate	321 (41)	355 (45)	3.4 (40.0)	9.5 (41.1)	53.2 (46.9)	55.3 (50.1)
Bayesian 24 h	320 (56)	354 (62)	2.9 (35.0)	8.9 (35.1)	46.5 (30.1)	47.4 (32.7)
Bayesian 48 h	333 (84)	368 (90)	16.2 (47.5)	23.2 (48.2)	65.0 (50.7)	68.0 (66.5)
Bayesian 72 h	323 (67)	358 (71)	5.9 (37.3)	12.7 (29.3)	49.8 (41.4)	40.9 (30.6)
Bayesian 96 h	322 (63)	356 (66)	4.6 (32.9)	10.6 (19.8)	43.9 (39.9)	28.3 (19.0)

Discussion

The use of our previously published 'expected range' for the steady-state phenobarbitone concentration to dose ratio gives accurate quantitative information on compliance in a population (Pullar *et al.*, 1989). However, although it also gives useful quantitative information concerning an individual's compliance (Kumar *et al.*,

1989a; Pullar *et al.*, 1988c, 1989) this is less accurate because the 50% variation covered by the expected range(s) coupled with the need to give individual patients the 'benefit of the doubt' may result in an overestimate of compliance. This is illustrated in Study 3, where three of eight volunteers with two thirds compliance had peak LDRs, and one both peak and trough LDRs, which fell within the expected range, although all had LDRs at least 20% lower than that predicted for full compliance using the 96 h Bayesian estimate and 8% lower using the initial estimate. We believe that the method described in this paper represents an improvement in the accuracy of the use of low-dose phenobarbitone to measure an individual's compliance.

Although no consistent statistical advantages were demonstrated for the initial estimate or any of the timed samples, the overall trend appeared to favour the estimate based on the 96 h post-single dose sample, especially in terms of precision. In no instance was the measured steady-state concentration greater than 13% different from that estimated using the 96 h post-single dose sample. In view of the small numbers involved, the fact that this appears least biased, most precise and has the smallest confidence intervals, and also makes the most 'kinetic sense' (as it is least affected by absorption or distribution), makes it the most appropriate choice for a single timed sample. This method might also be used to predict plasma drug concentrations before steady state is achieved without having to define new expected ranges. This would permit the use of phenobarbitone to measure compliance with limited (e.g. 10 days) courses of treatment.

Even the administration of a test dose with a single blood sample 96 h later might prove impractical in some situations and, in fact, might represent an undesirable

Figure 2 Steady-state peak and trough phenobarbitone concentration to dose ratios for the 8 subjects in Study 3. a) actual concentration to dose ratios with simulated poor (2/3) compliance (*), b) the actual steady-state concentration to dose ratios produced by daily dosing (○), c) the predicted concentration (initial estimate) assuming full compliance (+), and d) the predicted concentration (96 h Bayesian) to dose ratios assuming full compliance (●) are shown. The interrupted line represents the lower limit of the 'expected' range.

intervention which alerts the patient that their compliance is under scrutiny. However, in most cases, even the initial estimate derived solely from the population data appears to be accurate and for nine of the ten volunteers in Study 2 varied by no more than 17% from the actual steady-state concentration, although in the other it gave a value around one and a half-times the steady state concentration. While not perfect, the initial estimate may represent a reasonable compromise where a single dose study is impractical.

One caveat, at present, is that the predictive model described here was derived from and tested on young

healthy volunteers. However, the aim is to produce a model which will be sensitive to alterations in phenobarbitone elimination produced by factors such as age, disease or (occasionally) concurrent therapy. If the predictive model can be shown to remain accurate in these groups this will remove the need to produce 'normal ranges' for, and allow us to study, populations where such factors may be prevalent. Only the use of a model which includes some individual kinetic data from an affected individual, i.e. a post single-dose concentration, rather than one which uses only demographic details, is likely to be useful in such circumstances.

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