ELSEVIER

Archives of Medical Research 36 (2005) 49–53

Archives of Medical Research

ORIGINAL ARTICLE

Estimation of Population Pharmacokinetic Parameters of Free-Phenytoin in Adult Epileptic Patients

Leon Aarons,[a] Ikhlas Ali Ahmed[b] and Dirk Deleu[c]

[a]*School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, UK*
[b]*Department of Clinical Pharmacology and Therapeutics, Al Khod, Sultanate of Oman*
[c]*Department of Neurology (Medicine), Hamad Medical Corporation, Doha, Qatar*

Received for publication July 29, 2004; accepted October 29, 2004 (04/048).

Background. Serum concentrations of free-phenytoin (F-PHT) obtained in adult epileptic patients receiving PHT in monotherapy were analyzed to estimate the Michaelis–Menten pharmacokinetic parameters.

Methods. Steady-state F-PHT serum concentrations, PHT dosing history, and associated information were collected prospectively. The maximum metabolic rate (Vm) and Michaelis–Menten constant (Km) of F-PHT and their interindividual variability data were estimated using nonlinear mixed effects modeling (NONMEM).

Results. Twenty-nine patients with two or more available steady-state F-PHT serum concentrations (total of 63 dose/serum concentration pairs) met the inclusion criteria. Patients were taking PHT (100–500 mg/day) in monotherapy. The population estimates of F-PHT for Vm and Km were 9.1 mg/kg/day and 7.3 mg/L, respectively. The model was prospectively evaluated in a small group (seven) of additional patients.

Conclusions. The recommended daily dose in this population to achieve a F-PHT concentration of 1.5 mg/L is 6.1 mg/kg. © 2005 IMSS. Published by Elsevier Inc.

Key Words: Phenytoin, Epilepsy, NONMEM, Oman, Population pharmacokinetics, Protein binding, Michaelis–Menten kinetics.

Introduction

Phenytoin (PHT) is still one of the most important and widely prescribed anti-epileptic drugs (AED). Its efficacy together with its acceptable safety profile has made PHT one of the drugs of choice for generalized tonic-clonic and partial seizures at all ages. In addition, it still plays an invaluable role in the treatment of status epilepticus. Optimal therapy with PHT requires that its administration should be tailored to the needs of each individual patient. This is important because, at therapeutic concentrations, the pharmacokinetics of PHT are complicated by the nonlinear elimination due to capacity-limited liver metabolism (hydroxylation). Consequently, small increases in PHT dose can result in a disproportionate increase in serum PHT concentration and vice versa. In addition, genetically determined polymorphism of the hepatic microsomal enzyme system results in large interindividual and ethnic variations in the capacity to metabolize PHT (1). In addition to its nonlinear kinetics, the pharmacokinetics of PHT is affected by potential interactions with other AEDs with enzyme-inducing capacity (carbamazepine and phenobarbital). The therapeutic range of the unbound or free-phenytoin (F-PHT) serum concentration is about 10% of the total PHT serum concentration and varies from 3.3 to 9.6 μmol/L (0.83–2.27 mg/L) (2,3).

Mixed effects modeling together with the Bayesian feedback approach can be used to predict the daily dosage required to achieve desired steady-state serum concentrations (4,5). The NONMEM computer program can be used ideally for the estimation of population average values of pharmacokinetic parameters, such as maximum metabolic rate (Vm) and Michaelis–Menten constant (Km), their variability and the influence of demographic and clinical factors (covariates) on this variability (6). Furthermore, it has been shown that this approach is better than other methods with regard to

Address reprint requests to: Dirk Deleu, MD, PhD, FAAN, FRCP, Department of Neurology (Medicine), P.O. Box 3050, Hamad Medical Corporation, Doha, Qatar. Fax (974) 439 2377; E-mail: doc_deleu@hotmail.com

0188-4409/05 $–see front matter.
doi: 10.1016/j.arcmed.2004.11.006

precision and accuracy (6–8). In several studies this methodology has been applied to the estimation of population pharmacokinetic parameters for PHT (6,9–13).

The aim of this prospective data collection was to determine Vm and Km of PHT using F-PHT serum concentration data from routine monitoring of adult out-patients receiving PHT monotherapy for the management of epilepsy. Based on Bayesian forecasting these data may facilitate the selection and adjustment of PHT dosage regimens for individual patients in this population.

Patients and Methods

Patients

Patient data were collected from routine clinical visits between 1996 and 1998 at the Neurology Clinic of Sultan Qaboos University Hospital (SQUH) in the Sultanate of Oman, a well-equipped, tertiary care hospital receiving referrals from throughout the Sultanate. In this prospective study, all adult patients (age ≥18) with documented epilepsy diagnosis based on the basis of the definition proposed by the International League Against Epilepsy (ILAE) in 1993 (14) and taking the same oral brand of PHT (Epanutin® capsules) in monotherapy for more than 2 months were enrolled. The seizure types (generalized tonic-clonic or partial seizures) were identified on the basis of the classification proposed by the ILAE in 1981 (15). All patients responded well to PHT therapy (with a reduction in seizure frequency ≥50%) (16) and, in addition, were seizure free for the last 3 months.

The protocol was based on that of a previous population pharmacokinetic study (17). Briefly, exclusion criteria were pregnant and lactating females, patients with clinical or laboratory evidence of renal or hepatic disease, patients with drug or alcohol abuse or taking any concurrent medication known to interfere with PHT pharmacokinetics, and those patients with inaccurately documented dosage and sampling histories. To ensure optimal compliance, two to three measured serial F-PHT serum samples were taken at 4-week intervals on the same dose. A F-PHT serum concentration variation of 10% or less was regarded as acceptable. An initial screening was performed to include the patients for whom complete and reliable information was available.

Data collection included demographic data (age, gender and total body weight), PHT dosing history (total daily PHT dose, time of last PHT intake, dosing schedule), F-PHT serum concentration and F-PHT sampling time. The Medical Research and Ethics Committee approved the study and informed verbal consent was obtained from all patients.

Sampling and Drug Assay

To achieve steady-state conditions patients needed to be on a constant maintenance dosage for a minimum of 5 weeks (18). All blood samples for F-PHT serum concentration determination were obtained as part of routine monitoring therapy: all patients contributed at least two study samples at two different visits and all samples were taken a minimum of 9 h after the last intake of the drug so that the concentration reflects steady-state conditions. The number of dose–serum concentration pairs in Table 1 refers to the total number of F-PHT serum concentration samples collected corresponding with a PHT dose taken by the patient at that time (at steady state).

F-PHT fractions were obtained from the sera by ultrafiltration using the Centrifree Micropartition System (Millipore, Bedford, MA, USA) in a refrigerated centrifuge. One ml of serum was centrifuged (2500 rpm for 30 min) to prepare a protein-free ultrafiltrate (19). The F-PHT containing ultrafiltrates were then subsequently quantified using fluorescence polarization immunoassay (Abbott IMx System, Abbott Laboratories, Abbott Park, IL, USA) with inter- and intra-assay coefficient of variation on the F-PHT serum concentrations of <10% (20,21).

Pharmacostatistical Analysis

The F-PHT serum concentration-time profiles from all patients were analyzed with the NONMEM (version V) program to evaluate the population mean parameters (Vm and Km), and intra- and interindividual variability (22). At steady state, the dosing rate is equal to the elimination rate as shown below:

$$\frac{D}{\tau} = \frac{Vm \cdot Css}{Km + Css}$$

where D is the dose, τ is the dosing interval (24 h) and Css is the steady state concentration. Rearranging gives

$$Css = \frac{Km \cdot D}{Vm - D}$$

Table 1. Characteristics of the epileptic population studied

Parameters	Population
Number of patients	29
Gender (M/F)	19/10
Age (years)[a]	39.6 (15.3) (18–67)[b]
Weight (kg)[a]	70.0 (13.9) (52–107)[b]
Number of dose–serum concentration pairs	63
Number of samples per patient[c]	2
Dose (mg/day/kg)[a]	3.6 (1.4) (0.9–7.7)[b]
F-PHT C_{ss} (mg/L)[a]	0.57 (0.43) (0.05–2.17)[b]

[a]Values expressed as mean (SD).
[b]Range.
[c]Value expressed as median.
Abbreviation: F-PHT C_{ss}, free-phenytoin steady-state serum concentrations.

Inter- and intra-individual variability were modeled with multiplicative error terms. Least squares linear regression was used for preliminary analysis to examine the influence of age and body weight on the individual Michaelis–Menten parameters. Any significant patterns were investigated by mixed effects modeling (NONMEM).

Test Population

By using the population estimates of Vm and Km, an optimal dose that would result in a desired F-PHT serum concentration was calculated for seven patients, not belonging to the original patient population, and who needed dose adjustment. The additional patients consisted of five males and two females with an average age of 36.7 years (range 22–55 years) and average weight 75.6 kg (range 50–102 kg). Doses ranged from 200 to 500 mg per day. The predicted steady-state concentrations were compared with the observed concentrations after 4 weeks of dosage initiation.

Statistical Analysis

Where appropriate, data are expressed as means ± standard deviation (SD or SE). Student's *t*-test was used to examine the significance of any difference between the values from the study and test population. Probability values <0.05 were considered as statistically significant.

Results

Thirty-eight epileptic patients receiving PHT in monotherapy were screened, but eventually only 29 were enrolled; 20 with primary generalized tonic-clonic seizures and 9 had partial seizures with secondary generalization. Nine patients had exclusion criteria: one patient had clinical or laboratory evidence of hepatic or renal disease, three patients were taking concurrent medication other than AEDs, three patients had inaccurately documented dosage and sampling histories, and two were non-compliant. The clinical and demographic characteristics of the study population are summarized in Table 1. None of the patients experienced major adverse drug reactions requiring discontinuation or dose reduction.

The F-PHT pharmacokinetic parameters were 634 (±51) mg/day and 0.73 (±0.23) mg/L for Vm and Km, respectively. The interindividual variability for Km was 21 (±12)%. No estimate could be obtained for Vm. Intraindividual variability was estimated to be 22 (±5)%. Assuming that PHT is 90% bound to plasma proteins (3), the Km value for total PHT would be equal to 7.3 mg/L. Vm showed no correlation with weight or any of the other covariates. Similarly, Km correlated very poorly with age and weight and was independent of sex. A plot of Css vs. dose is shown in Figure 1.

The mean F-PHT concentration in the test group was 0.76 (SE 0.13) mg/L and the mean predicted concentration was 0.89 (SE 0.23) mg/L. There was no significant difference between the observed and predicted concentration (*t*-value 0.47; $p = 0.64$) and the correlation coefficient between the two set of concentrations was 0.57.

Discussion

The unique pharmacokinetic profile makes PHT difficult to use without the help of drug monitoring. Although numerous nomograms (23,24) and graphic estimation (25,26) have been proposed to help the clinician in facilitating attainment of optimal individualized dosage for PHT, these methods are highly dependent on the following: a) the accuracy and precision of the population pharmacokinetic parameters used to construct them and b) the invalid assumptions of similarity between the study population and the patients for whom the nomogram is to be used (constant Vm or Km). Therefore in the individualization of PHT dosage, it is important to use estimates of Vm and Km obtained in a patient population in whom the drug is used clinically (5,6).

This study differs from other pharmacokinetic studies of PHT in that: a) it is the first study in which the Vm and Km estimates of F-PHT are determined; b) it is the first population pharmacokinetic study on PHT in this population. Since our study population was representative for this population, the results of this study can be implemented in PHT prescribing and facilitate the physician's task in dose optimization for individual patients during chronic PHT therapy. Although the exclusion of patients taking concurrent antiepileptic medication could be considered as a limitation, in our experience the majority (63%) of patients in our population taking PHT can be completely or optimally controlled on PHT monotherapy (27).

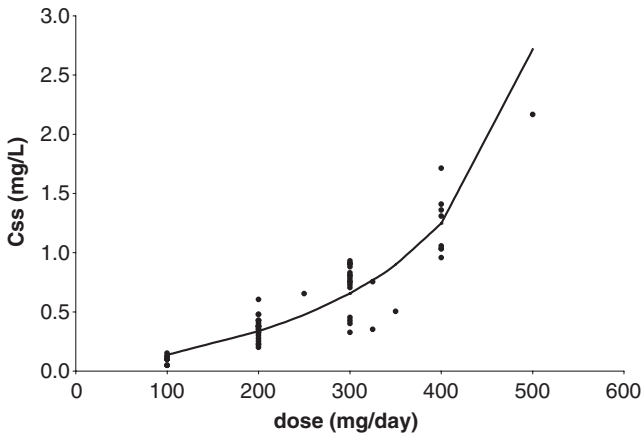

Figure 1. Plot of steady state F-PHT concentration versus daily dose. The solid line shows the fitted model.

Although the PHT dose-serum concentration relationship is complicated, serum concentrations are still used clinically. Several studies have shown the benefit of monitoring F-PHT serum levels in reducing toxicity to a minimum (28). In addition to genetic influence (29,30), age, sex and body weight, smoking, disease states and concurrent drug therapy have been shown to be important determinants of the serum concentration-dose relationship for PHT (12,31,32). Any change in dosage should therefore be accompanied by careful patient monitoring. In our study none of the patients were smokers.

The reported ranges for Vm and Km are 100 to 1000 mg/day and 1 to 15 mg/L, respectively, due to large interindividual and interethnic variability in these values, and consequently the concentration at which metabolism becomes saturated is difficult to predict (8,9). On the basis of our estimated values of Km and Vm, the average dose required to achieve a PHT serum concentration of 1.5 mg/L would be 6.09 mg/kg/day (based on an average weight of 70 kg). Note that in our study, weight did not statistically explain any of the variability in the pharmacokinetic parameters and the recommended dose is expressed per kg so that it can be compared with other studies, which report similar values (8,33). Certain studies indicate an inverse relationship between Vm with age (13) and a positive correlation with weight (9,13), while Km was affected by concurrent drug therapy (34). In contrast with these observations, we did not find any influence of the covariates (age, weight, and gender) on Vm or Km. The relatively small sample of our study could perhaps account for this. The values of Vm and Km for PHT determined in our population are slightly higher than those reported in other populations (Table 2), although any comparison must be tentative given the small number of patients in our study. A small scale pharmacokinetic study performed in adult epileptic patients in Saudi Arabia using graphical methods to determine Michaelis–Menten parameters of PHT revealed values of Vm and Km equal to 8.0 mg/kg/day and 6.5 mg/L, respectively (35). The difference between these Michaelis–Menten pharmacokinetic parameters in this Saudi epileptic population and our population is probably based on interethnic differences and indicates that disposition of PHT between ethnic groups can be very variable.

Table 2. Population pharmacokinetics of total phenytoin showing ethnic variations in phenytoin metabolizing capacity

Population	Vm (mg/kg/day)	Km (mg/L)	Author (reference)
African (Black)	6.5	3.4	Miller et al. 1987 (11)
Chinese	7.3	6.2	Rui et al. 1982 (37)
European	5.9	5.7	Grasela et al. 1983 (9)
Japanese	5.4	2.4	Yukawa et al. 1990 (13)
Malaysian	7.3	3.7	Ismail et al. 1994 (33)
Omani	9.1	7.3	Deleu et al. (present study)
Saudi	8.0	6.5	Abduljabbar et al. 1999 (35)

Members of the cytochrome P450 superfamily (CYP2C9 and CYP2C19) play a major role in the metabolism of PHT, and proved to be polymorphic as a result of single nucleotide polymorphisms, gene deletions, and gene duplications (36). Table 2 shows that Japanese epileptic patients more easily saturate phenytoin metabolism because of their lower Km value, than Omani patients. However, Chinese patients do not look that different in this respect. The values for Vm and Km in the epileptic population on the Arabian peninsula (Saudi Arabia and Oman) seem to be the highest reported so far (Table 2).

In conclusion, we studied the pharmacokinetics of F-PHT in this adult epileptic out-patient population using steady-state serum concentration data obtained from routine monitoring. Further studies will indicate whether these values are useful in clinical practice for the selection of the required individual daily dose of PHT. However the initial 'validation' study in a small group of patients was encouraging.

Acknowledgments

This study was supported by Sultan Qaboos University (SQU) grant No. IG/MED/PHAR 99/01. We are grateful to Mrs. H. Adnan, Mr. C. Batchelor, and Mrs. H. Due-Boje of the Clinical Biochemistry Lab, SQU Hospital.

References

1. Andoh B, Idle JR, Sloan TP, Smith RL, Woolhouse N. Inter-ethnic and inter-phenotype differences among Ghanaians and Caucasians in the metabolic hydroxylation of phenytoin. Br J Clin Pharmacol 1980; 9:282P–283P.
2. Bryson SM, Al-Lanqawi Y, Kelman AW, Whiting B. Comparison of a Bayesian forecasting technique with a new method for estimating phenytoin dose requirements. Ther Drug Monit 1988;10:80–84.
3. Levine M, Chang T. Therapeutic drug monitoring of phenytoin: rationale and current status. Clin Pharmacokinet 1990;19:341–358.
4. Peterson GM, MacLean S, Aldous S, Von Witt RJ, Millingen KS. Plasma protein binding of phenytoin in 100 epileptic patients. Br J Clin Pharmacol 1982;14:298–300.
5. Vozeh S, Muir KT, Sheiner LB, Follath F. Predicting individual phenytoin dosage. J Pharmacokinet Biopharm 1981;9:131–146.
6. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetics parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. J Pharmacokinet Biopharm 1980;8:553–571.
7. Burton ME, Vasko MR, Brater DC. Comparison of drug dosing methods. Clin Pharmacokinet 1985;10:1–37.
8. Yuen GJ, Taylor JW, Ludden TM, Murphy MJ. Predicting phenytoin dosages using Bayesian feedback: a comparison with other methods. Ther Drug Monit 1983;5:437–441.
9. Grasela TH, Sheiner LB, Rambeck B, Boenigk HE, Dunlop A, Mullen PW, Wadsworth J, Richens A, Ishizaki T, Chiba K. Steady-state pharmacokinetics of phenytoin from routinely collected patient data. Clin Pharmacokinet 1983;8:355–364.
10. Chan E, Ti TY, Lee HS. Population pharmacokinetics of phenytoin in Singapore Chinese. Eur J Clin Pharmacol 1990;39:177–181.
11. Miller R, Rheeders M, Klein C, Suchet I. Population pharmacokinetics of phenytoin in South African black patients. S Afr Med J 1987;72: 188–190.

12. Valodia P, Seymour MA, Miller R, McFadyen ML, Folb PI. Factors influencing the population pharmacokinetic parameters of phenytoin in adult epileptic patients in South Africa. Ther Drug Monit 1999;21: 57–62.
13. Yukawa E, Higuchi S, Aoyama T. Population pharmacokinetics of phenytoin from routine clinical data in Japan: an update. Chem Pharm Bull (Tokyo) 1990;38:1973–1976.
14. Commission on Epidemiology and prognosis, International League Against Epilepsy. Guideliness for epidemiologic studies on epilepsy. Epilepsia 1993;34:592–596.
15. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 1981;22: 489–501.
16. Birbeck GL, Hays RD, Cui X, Vickrey BG. Seizure reduction and quality of life improvements in people with epilepsy. Epilepsia 2002; 43:535–538.
17. Deleu D, Aarons L, Ahmed I. Population pharmacokinetics of free-carbamazepine in adult Omani epileptic patients. Eur J Clin Pharmacol 2001;57:243–248.
18. Yukawa E. Optimisation of antiepileptic drug therapy. The importance of serum drug concentration monitoring. Clin Pharmacokinet 1996; 31:120–130.
19. Argyle JC, Kinniburgh DW, Costa R, Jennison T. Evaluation of an ultrafiltration-fluorescence polarization immunoassay for monitoring unbound phenytoin. Ther Drug Monit 1984;6:117–120.
20. Loomis KF, Frye RM. Evaluation of the Abbott TDx for the stat measurement of phenobarbital, phenytoin, carbamazepine, and theophylline. Am J Clin Pathol 1983;80:686–691.
21. Gurley BJ, Marx M, Olsen K. Phenytoin free fraction determination: comparison of an improved direct serum injection high-performance liquid chromatographic method to ultrafiltration coupled with fluorescence polarization immunoassay. J Chromatogr B Biomed Appl 1995; 670:358–364.
22. Boeckmann AJ, Sheiner LB, Beal SL. NONMEM Users guide. University of California, San Fransisco;1994.
23. Richens A, Dunlop A. Serum-phenytoin levels in management of epilepsy. Lancet 1975;2:247–248.
24. Rambeck B, Boenigk HE, Dunlop A, Mullen PW, Wadsworth J, Richens A. Predicting phenytoin dose—a revised nomogram. Ther Drug Monit 1979;1:325–333.
25. Robinson FC, Mayer PR. A graphic method for predicting individual phenytoin levels in an office practice. Ther Drug Monit 1982;4:225–228.
26. Armijo AJ, Cavada E. Graphic estimation of phenytoin dose in adults and children. Ther Drug Monit 1991;13:507–510.
27. Hanssens Y, Deleu D, Al Balushi K, Al Hashar A, Al Zakwani I. Drug utilization pattern of antiepileptic drugs: a pharmacoepidemiologic study in Oman. J Clin Pharm Ther 2002;27:357–364.
28. Peterson GM, Khoo BH, von Witt RJ. Clinical response in epilepsy in relation to total and free serum levels of phenytoin. Ther Drug Monit 1991;13:415–419.
29. Eichelbaum M. Defective oxidation of drugs: pharmacokinetic and therapeutic implications. Clin Pharmacokinet 1982;7:1–22.
30. Watanabe M, Iwahashi K, Kugoh T, Suwaki H. Phenytoin kinetics in Japanese adult epileptics: phenotype of phenytoin slow metabolizers. Clin Neuropharmacol 1997;20:346–351.
31. Sherwin AL, Loynd JS, Bock GW, Sokolowski CD. Effects of age, sex, obesity, and pregnancy on plasma diphenylhydantoin levels. Epilepsia 1974;15:507–521.
32. Richens A. Clinical pharmacokinetics of phenytoin. Clin Pharmacokinet 1979;4:153–169.
33. Ismail R, Rahman AF, Chand P. Pharmacokinetics of phenytoin in routine clinic patients in Malaysia. J Clin Pharm Ther 1994;19:245–248.
34. Bauer LA, Blouin RA. Age and phenytoin kinetics in adult epileptics. Clin Pharmacol Ther 1982;31:301–304.
35. Abduljabbar M, Al-Khamis K, Ogunniyi A, Daif AK, Al-Yamani M. Phenytoin dosage adjustment in Saudi epileptics: utilization of steady-state pharmacokinetic parameters. Eur J Neurol 1999;6:331–334.
36. Bjornsson TD, Wagner JA, Donahue SR, Harper D, Karim A, Khouri MS, Murphy WR, Roman K, Schneck D, Sonnichsen DS, Stalker DJ, Wise SD, Dombey S, Loew C. A review and assessment of potential sources of ethnic differences in drug responsiveness. J Clin Pharmacol 2003;43:943–967.
37. Rui JZ, Cai MH, Chu XM, Chen G. NONMEM approach for estimating population pharmacokinetic parameters of phenytoin in Chinese epileptics. Yao Xue Xue Bao 1995;30:172–178.