Population Pharmacokinetics of Phenytoin from Routine Clinical Data in Japan: An Update

Eiji YUKAWA,* Shun HIGUCHI, and Toshinobu AOYAMA

Department of Hospital Pharmacy, Kyushu University Hospital, Faculty of Medicine, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received November 2, 1989*

Routine clinical pharmacokinetic data collected from outpatients receiving phenytoin (PHT) were reanalysed to estimate population pharmacokinetic parameters. There were 756 steady-state PHT concentrations and associated dosage rates (mg/d) from 334 outpatients. The data were analysed using nonlinear mixed effects model (NONMEM), a computer program designed for population pharmacokinetic analysis that allows pooling of data from many individuals. The influence of weight, co-anticonvulsants on the maximum elimination rate (V_m) and age, co-anticonvulsants on the Michaelis–Menten constant (K_m) and the influence of dosage form on the bioavailability (F) of PHT were investigated.

The V_m and K_m of a 60 kg adult outpatient treated with PHT alone were estimated to be 325 mg/d and 2.41 μg/ml, respectively, while for a same size individual taking PHT with co-anticonvulsants respective estimates were 351 mg/d and 3.18 μg/ml. The parameter of a power function of weight was estimated to adjust V_m for body size. The best function adjusts V_m in proportion to weight to the 0.737 power. The K_m for patients less than 15 years old was 24.8% less than that of adults. Assuming the F of PHT to be 1 in patients prescribed a tablet, the F value in patients prescribed a powder was $[1-\exp(-9.92/D_{ij})]$; D_{ij} is the daily dose of PHT for the ith C_{pss} in the jth patients (mg/kg/d).

Keywords population pharmacokinetics; phenytoin; NONMEM; Michaelis–Menten

Introduction

Phenytoin (PHT) is a widely prescribed anticonvulsant for epileptic patients of all ages. The therapeutic range is narrow with concentrations of 5 to 20 μg/ml in children[1,2] and 10 to 20 μg/ml in adults.[3,4] Achieving this range with standard dosage regimens is made more difficult by the presence of non-linearity of PHT pharmacokinetics and large intersubject differences in kinetic parameters. Consequently, a small change in PHT dosage may produce a disproportionately large change in steady-state serum concentration. In the design of a PHT dosage regimen it is therefore important to use values of pharmacokinetic parameters which are representative of the population.

Recently, several investigators[5-7] have reported average values of the Michaelis–Menten parameters and their variability from routine clinical pharmacokinetic data using the nonlinear mixed effects model (NONMEM) approach.[8-10] We have also reported the successful application of the NONMEM approach in the estimation of Michaelis–Menten pharmacokinetic parameters from routine clinical data of 220 Japanese outpatients.[11] But, until now, as far as we are aware, an evaluation of the influence of co-anticonvulsants on these parameters and the relative bioavailability of powder form decrease with an increase of dose has not been undertaken.[12,13] Accordingly, we investigated the influence of these factors on Michaelis–Menten pharmacokinetic parameters in a large population visiting the epilepsy outpatient clinic at kyushu University Hospital, using data gathered in the routine care of the patients.

Materials and Methods

Data Sources We retrospectively selected 334 outpatients (170 males and 164 females) in Kyushu University Hospital who had two or more reliable measurements of the steady-state concentration of PHT in serum, made while they were taking different daily doses. Patients in whom concurrent therapy was altered were excluded from the study. Those studied were taking PHT alone (101 patients) or PHT combined with other anticonvulsants (233 patients) (Table I). Their ages and weights ranged from 0.6 years to 71.1 (mean 24.3 years, S.D. 14.1 years) and 9.0 kg to 115.0 (mean 49.1 kg, S.D. 15.5 kg). Table II shows the pertinent characteristics of the patient population. The frequency distribution within the data set of their demographic factors, age, weight, daily dose and serum concentration are displayed in Fig. 1. All patients had normal renal and hepatic function, and were given PHT acid (Aleviatin® brand tablets and powders [Dainippon Pharmaceutical Co., Ltd., Osaka]). Four steady-state serum concentrations at four different doses were determined in 11 patients, three steady-state serum concentrations at three different doses were determined in 66 patients and two steady-state serum concentrations at different doses were determined in 257 patients. PHT was prescribed two to three times a day as a tablet or a powder preparation. The concentration of PHT was determined at least 30 d after any change in dosage. This time interval between changes in dosage was considered adequate to reach a new steady-state concentration in the serum. All blood samples were drawn at approximately 2—5 h after administration of a dose. The PHT concentration was routinely measured by immunoassay

TABLE I. Medications

Combined drugs	Number of patients
PHT alone	101
PHT+PB	45
PHT+CBZ	29
PHT+VPA	15
PHT+PB+CBZ	32
PHT+PB+VPA	14
PHT+CBZ+VPA	9
PHT+PB+CBZ+VPA	25
PHT+PB, CBZ, VPA+other drugs	64
Total	334

PHT = phenytoin, PB = phenobarbital, CBZ = carbamazepine, VPA = valproate sodium. Other drugs = primidone, clonazepam, sultiame, ethotoin, ethosuximide, acetazolamide, diazepam.

TABLE II. Summary of Data from Patients Treated with PHT

Characteristic	
Number of patients[a,b]	334
Number of observations[c]	756
Means and standard deviations	
Age (years)	24.3±14.1
Weight (kg)	49.1±15.5
Daily dose, R (mg/d)	225.8±73.1
Steady-state concentration, C_{ss} (μg/ml)	9.78±7.77

a) Males: 170, females: 164. *b*) Monotherapy: 101; polytherapy: 233. *c*) Observations of patients treated by tablets numbered 413.

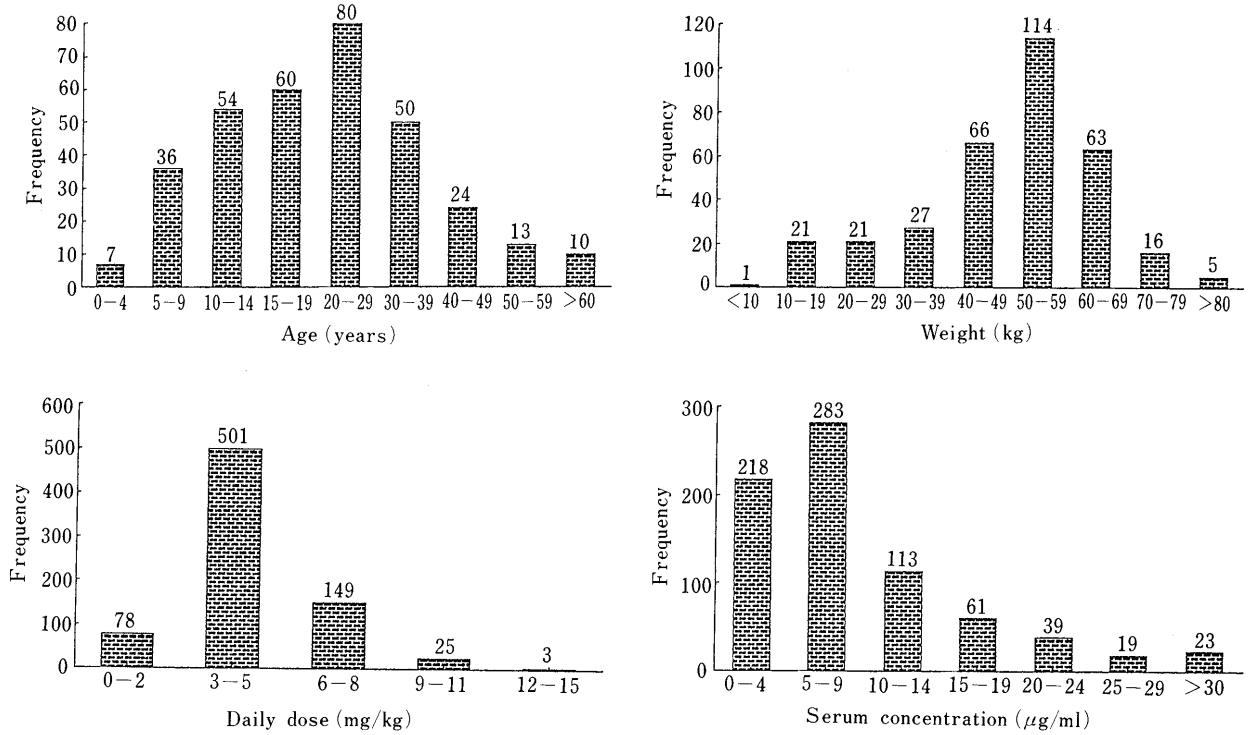

Fig. 1. Frequency Distribution of Age, Weight, Daily Dose and Serum Concentration from Patients Data

techniques (EMIT® or FPIA). The coefficient of variation of this assay was less than 10%.

Pharmacokinetic Model The Michaelis–Menten model is used to describe the pharmacokinetics of PHT.

$$R_{ij}=\frac{V_{mij}\cdot C_{pssij}}{F_{ij}\cdot(K_{mij}+C_{pssij})} \tag{1}$$

where R_{ij} is the daily dose of PHT for the ith C_{pss} in the jth patient (mg/d), V_{mij} is the ith maximum elimination rate (mg/d) for the jth patient, k_{mij} is the ith Michaelis–Menten constant (μg/ml) for the jth patient, C_{pssij} is the ith steady-state concentration (μg/ml) for the jth patient, and F_{ij} is the bioavailability of PHT for the ith dosage form administered to the jth patient.

We examined the influence of weight and co-anticonvulsants on the population mean values for V_m of PHT using Eq. 2, and examined the influence of age and co-anticonvulsants on the population mean values for K_m of PHT using Eq. 3. The influence of powder on the bioavailability of PHT was determined by Eq. 4. We considered the constant value of BA_{ij} in model 1, the exponential decrease of BA_{ij} with an increase of dose (D_{ij}; mg/kg/d) in model 2, and the linear decrease of BA_{ij} with an increase of dose in model 3.

$$\hat{V}_{mij}=V_m\cdot(WGT_{ij}/60)^{\theta_{Pw}}\cdot CO_{ij}{}^{Vm} \tag{2}$$

$$\hat{K}_{mij}=K_m\cdot AGE_{ij}\cdot CO_{ij}{}^{Km} \tag{3}$$

$$F_{ij}=BA_{ij} \tag{4}$$

$$AGE_{ij}=\begin{cases}1 & \text{if age}\geqq 15\\ \theta_{AGE} & \text{if age}<15\end{cases} \tag{5}$$

$$CO_{ij}{}^{Vm}=\begin{cases}1 & \text{for monotherapy}\\ \theta_{CO}{}^{Vm} & \text{for polytherapy}\end{cases} \tag{6}$$

$$CO_{ij}{}^{Km}=\begin{cases}1 & \text{for monotherapy}\\ \theta_{CO}{}^{Km} & \text{for polytherapy}\end{cases} \tag{7}$$

$$BA_{ij}=\begin{cases}1 & \text{for tablet}\\ \theta_{BA1} & \text{for powder (model 1)}\end{cases} \tag{8}$$

$$BA_{ij}=\begin{cases}1 & \text{for tablet}\\ 1-e^{-\theta_{BA2}/D_{ij}} & \text{for powder (model 2)}\end{cases} \tag{9}$$

$$BA_{ij}=\begin{cases}1 & \text{for tablet}\\ 1-\theta_{BA3}\cdot D_{ij} & \text{for powder (model 3)}\end{cases} \tag{10}$$

where $\hat{V}_{mij}$, $\hat{K}_{mij}$, and F_{ij} are the ith predicted parameters for the jth individual, respectively; V_m and K_m are the parameter values for the 'standard' patient (adult, weight 60 kg, PHT alone, tablet); WGT_{ij} is the ith weight of the jth individual in kg; θ_{Pw} is the power of weight for size adjustment of V_m; AGE_{ij} is an indicator variable which has a value of unity if the jth patient is more than 15 years old, and θ_{AGE} otherwise; $CO_{ij}{}^{Vm}$ or $CO_{ij}{}^{Km}$ are indicator variables which have a value of unity if the jth patient is treated with PHT alone, and $\theta_{CO}{}^{Vm}$ or $\theta_{CO}{}^{Km}$ otherwise, respectively; BA_{ij} is an indicator variable which has a value of unity if the PHT of the jth patient is prescribed as a tablet, and θ_{BA1} in model 1, $[1-\exp(-\theta_{BA2}/D_{ij})]$ in model 2 or $[1-\theta_{BA3}\cdot D_{ij}]$ in model 3 otherwise, D_{ij} is the daily dose of PHT for the ith C_{pss} in the jth patient (mg/kg/d).

θ_{AGE}, θ_{Pw}, θ_{BA1}, θ_{BA2}, θ_{BA3}, $\theta_{CO}{}^{Vm}$ and $\theta_{CO}{}^{Km}$ represent the fractional increase or decrease in the pharmacokinetic parameter associated with the presence of the corresponding indicator variable.

Statistical Model The statistical model must account for interindividual variation in V_m and K_m and for intraindividual error. The latter represents uncertainty in the relationship between the daily dosage predicted by Eq. 1 and the observed daily dosage.

In modeling the uncertainty between observed and predicted values we assume

$$\ln R_{ij}=\ln \hat{R}_{ij}+\varepsilon_{ij} \tag{11}$$

where $\hat{R}_{ij}$ is the predicted daily dosage of PHT for the ith R-C_{pss} pair in the jth patient (mg/d), the ε_{ij} is independent identically distributed statistical errors with mean zero and variance $\sigma_E{}^2$.

For interindividual variation, we assume

$$\ln(V_{mij})=\ln(\hat{V}_{mij})+\eta_j{}^{Vm} \tag{12}$$

$$\ln(K_{mij})=\ln(\hat{K}_{mij})+\eta_j{}^{Km} \tag{13}$$

where $\hat{V}_{mij}$ and $\hat{K}_{mij}$ are from Eq. 2 and 3, $\eta_j{}^{Vm}$ and $\eta_j{}^{Km}$ are independently distributed statistical errors with mean zero and variances ω^2_{Vm} and ω^2_{Km}.

Data Analysis Analysis was performed with the NONMEM computer program developed by Beal and Sheiner[8] on the mainframe computer of Kyushu University (FACOM M-780). The program uses the method of extended least squares to estimate population pharmacokinetic parameters. Using NONMEM, one analyses all the data together, but properly adjusts for the correlation among observations within each individual. NONMEM provides estimates of all population parameters and standard errors for all, including the parameters $\sigma_E{}^2$, $\omega_{Vm}{}^2$ and $\omega_{Km}{}^2$. To test the hypothesis whether the fit of the model to the data was significantly different, we used the value of the extended sum of squares (*ESS*), which is the minimum

objective function determined in the NONMEM fitting routine. Assuming that interindividual and intraindividual variance are normally distributed, then the difference in *ESS* is distributed as chi-square with degrees of freedom equal to the number of parameters that are fixed to hypothesized values. In all statistical tests $p<0.05$ was considered as statistically significant.

Results

Routine clinical pharmacokinetic data collected from outpatients receiving PHT were analysed to estimate population pharmacokinetic parameters. There were 756 steady-state PHT concentrations and associated dosage rates (mg/d) from 334 outpatients.

The results of our data analyses in model 1 indicated that the V_m and K_m of a 60 kg adult outpatient treated with PHT alone were 322 mg/d and 2.68 μg/ml for the patient population studied (Table III). The typical magnitude of the interindividual variability, as expressed by the coefficient of variation, was 17.2% for the V_m and 53.3% for the K_m. The typical magnitude of the intraindividual variability, as expressed by the coefficient of variation, was 11.0%. The V_m and K_m of a 60 kg adult outpatient taking PHT with co-anticonvulsants were estimated to be 361 mg/d and 3.75 μg/ml, respectively. NONMEM estimates indicated that a non-linear function of weight ($WGT^{0.543}$) as the optimum adjustment of V_m for body size is preferable to a linear function. The K_m for patients less than 15 years old was 13.7% less than that of adults ($K_m=2.31$ or 3.24 μg/ml). Assuming the F of PHT to be 1 in patients prescribed a tablet, the F value in patients prescribed a powder was 0.84.

The results of our data analyses in model 2 indicated that the V_m and K_m of a 60 kg adult outpatient treated with PHT alone were 325 mg/d and 2.41 μg/ml for the patient population studied (Table III). The typical magnitude of the interindividual variability, as expressed by the coefficient of variation, was 19.3% for the V_m and 63.1% for the K_m. The typical magnitude of the intraindividual variability, as expressed by the coefficient of variation, was 10.3%. The V_m and K_m of a 60 kg adult outpatient taking PHT with co-anticonvulsants were estimated to be 351 mg/d and 3.18 μg/ml, respectively. NONMEM estimates indicated that a non-linear function of weight ($WGT^{0.737}$) as the optimum adjustment of V_m for body size is preferable to a linear function. The K_m for patients less than 15 years old was 24.8% less than that of adults ($K_m=1.81$ or 2.39 μg/ml). Assuming the F of PHT to be 1 in patients prescribed a tablet, the F value in patients prescribed a powder was $[1-\exp(-9.92/D_{ij})]$.

The results of our data analyses in model 3 indicated that the V_m and K_m of a 60 kg adult outpatient treated with PHT alone were 326 mg/d and 2.59 μg/ml for the patient population studied (Table III). The typical magnitude of the interindividual variability, as expressed by the coefficient of variation, was 18.9% for the V_m and 59.1% for the K_m. The typical magnitude of the intraindividual variability, as expressed by the coefficient of variation, was 10.3%. The V_m and K_m of a 60 kg adult outpatient taking PHT with co-anticonvulsants were estimated to be 355 mg/d and 3.39 μg/ml, respectively. NONMEM estimates indicated that a non-linear function of weight ($WGT^{0.693}$) as the optimum adjustment of V_m for body size is preferable to a linear function. The K_m for patients less than 15 years old was 21.3% less than that of adults ($K_m=2.04$ or 2.67 μg/ml). Assuming the F of PHT to be 1 in patients prescribed a tablet, the F value in patients prescribed a powder was $[1-0.0325\cdot D_{ij}]$.

The small standard errors in Table III indicate a good fit with reliable parameter estimates for each model. Therefore, the prediction abilities in determination of the initial maintenance dose were compared with each model. Population parameters estimated were used to predict the dosage of PHT that would be expected to produce the desired concentration. The predicted dosage was compared with the actual dosage, and the bias (mean prediction error: ME) and precision (mean absolute prediction error: MAE) were determined. The ME is the mean of the predicted dosage minus the actual dosage.

The predictive performances in predicting PHT dosage using each model were obtained similarly, but the MAE of model 2 was superior to that of the other models (Table

TABLE III. Final Parameter Estimates in Each Model

Parameter	Medel 1 Estimate	(SEM)	Model 2 Estimate	(SEM)	Model 3 Estimate	(SEM)
V_m (mg/d/ standard individual)	322	(8.30)	325	(9.18)	326	(8.92)
K_m (μg/ml)	2.68	(0.28)	2.41	(0.25)	2.59	(0.26)
ω_{Vm} (%)	17.2[a]	(2.4)[b]	19.3[a]	(2.2)[b]	18.9[a]	(2.2)[b]
ω_{Km} (%)	53.3[a]	(5.3)[b]	63.1[a]	(5.1)[b]	59.1[a]	(4.9)[b]
σ_E (%)	11.0[a]	(0.5)[b]	10.3[a]	(0.5)[b]	10.3[a]	(0.5)[b]
θ_{Pw}	0.543	(0.04)	0.737	(0.036)	0.693	(0.039)
θ_{AGE}	0.863	(0.076)	0.752	(0.073)	0.787	(0.074)
θ_{CO}^{Vm}	1.12	(0.039)	1.08	(0.039)	1.09	(0.039)
θ_{CO}^{Km}	1.40	(0.167)	1.32	(0.165)	1.31	(0.155)
θ_{BA1}	0.840	(0.017)	—	—	—	—
θ_{BA2}	—	—	9.92	(0.52)	—	—
θ_{BA3}	—	—	—	—	0.0325	(0.002)
ESS[c]	6309.013		6211.026		6227.661	

a) All estimates of the variance components are expressed as coefficients of variation. *b*) SEM of the variances (σ^2 or ω^2) is estimated by NONMEM. The SEM of the standard deviation ($\sqrt{\sigma^2}$ or $\sqrt{\omega^2}$) was approximated by $SEM_{S.D.}=\sqrt{\sigma^2+SEM_{var.}}-\sqrt{\sigma^2}$ and expressed in % of $\sqrt{\sigma^2}$. *c*) Value of objective function estimated by NONMEM.

TABLE IV. Performance in PHT Dosage Predictions

Model	n	Correlation[a] coefficient (r)	ME (95% c.i.)[b] (mg/d)	MAE (95% c.i.) (mg/d)
1	756	0.755	−0.90 (−4.32 to 2.53)	35.97 (33.69 to 38.25)
2	756	0.801	−1.21 (−4.34 to 1.92)	32.47 (30.37 to 34.57)
3	756	0.789	−0.51 (−3.72 to 2.70)	33.46 (31.31 to 35.61)

a) Correlation coefficient between the actual and predicted dose. *b*) 95% confidence intervals of the mean.

TABLE V. Influence of Various Factors in Model 2

Parameter	Hypothesised value[a]	NONMEM estimate[b]	log likelihood difference	p value
θ_{Pw}	1	0.737	72.35	$p<0.005$
θ_{AGE}	1	0.752	13.67	$p<0.005$
θ_{CO}^{Vm}	1	1.08	5.38	$p<0.05$
θ_{CO}^{Km}	1	1.32	5.89	$p<0.02$
θ_{BA2}	—	9.92	195.71	$p<0.005$

a) One parameter had to be a hypothesised value. *b*) All parameters were included and estimated.

IV). Therefore, the extent of PHT absorption from powder form was proved to be exponentially decreased with an increase of dose (D_{ij}; mg/kg/d) in a usual dose for children and adults.

Table V summarizes the results of the hypothesis testing of model 2 investigating the influence of characteristics of different patients on the pharmacokinetics parameters. The V_m and K_m are significantly increased in patients taking PHT with co-anticonvulsants ($\theta_{CO}{}^{Vm} \neq 1$, $p<0.05$; $\theta_{CO}{}^{Km} \neq 1$, $p<0.02$). K_m is significantly reduced in patients less than 15 years of age ($p<0.005$). NONMEM estimates indicated that a non-linear function of weight ($WGT^{0.737}$) as the optimum adjustment of V_m for body size is preferable to a linear function ($p<0.005$). The estimate of F of PHT in patients prescribed a powder was significantly lower than of PHT in patients prescribed a tablet ($p<0.005$).

Discussion

PHT dosing for individual patients can be viewed as the following process: a) determination of the initial maintenance dose using the average values of pharmacokinetic parameters, b) a first dosage adjustment using a feedback control method (*i.e.*, Bayesian feedback technique) based on one dose-steady-state serum concentration pair, and c) dosage adjustments based on two or more dose-steady-state serum concentration pairs. The population pharmacokinetic parameters have an important role in current therapeutic drug monitoring. The NONMEM system has been employed to obtain estimates of the population pharmacokinetic parameters from serum concentration measurements made in patients during routine treatment.

The results of this analysis suggest that the V_m of PHT is adequately described using a non-linear function of weight together with the report of Grasela *et al.*[6] Some investigators have proposed a scaling factor to relate metabolic activity to body size.[14] Body surface area or weight raised to the power of 0.7 has been used to adjust the maintenance dose of PHT.[15] The 0.737 power noted in V_m (corrected for size) was similar to that. The V_m for patients taking PHT combined with other anticonvulsants was 8% higher thant that for patients taking PHT alone, whereas the K_m for patients taking PHT combined with other anticonvulsants was 32% higher than that for patients taking PHT alone. These results suggest that patients taking PHT combined with another anticonvulsant have a more mild saturable hepatic metabolism at clinical achievable concentrations than do patients taking PHT alone.

Since PHT is a poorly water-soluble and weak acid with a pK_a of 8.3, problems with gastrointestinal dissolution and subsequent absorption into the systemic circulation are anticipated. PHT powder is widely used in Japan, and the extent of PHT absorption in this form has been proved to be not only lower than when taken in tablet form, but also to decrease with an increase in the usual dose.[12,13] The cause is thought to be the lower wettability of PHT powder. The final regression model for PHT absorption from powder form suggested its exponential reduction with dosage increase. However, the percent of relative bioavailability of PHT powder was nearly 100% in less than 2 mg/kg of daily dose (Fig. 2), suggesting that the dissolution rate of this powder is equivalent to that of tablets up to about 2 mg/kg of daily dose.

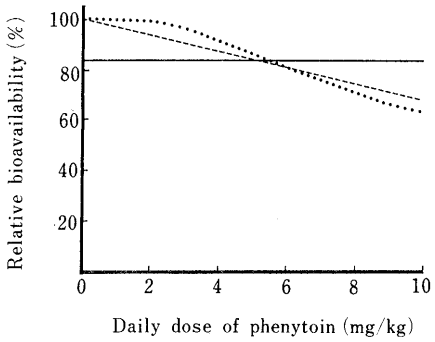

Fig. 2. Relationship between Daily Dose of PHT and Relative Bioavailability from PHT Powders

——, model 1; · · · ·, model 2; ------, model 3.

Both V_m and K_m values of our analysis were lower than those presented by other investigators [in patients taking PHT alone $\{V_m = 15.9 \times (\text{weight})^{0.737}$ mg/d; $K_m = 2.41$ μg/ml for ≧15 yr.; $K_m = 1.81$ μg/ml for <15 yr.$\}$, and in patients taking PHT combined with other anticonvulsants $\{V_m = 17.2 \times (\text{weight})^{0.737}$ mg/d; $K_m = 3.18$ μg/ml for ≧15 yr.; $K_m = 2.39$ μg/ml for <15 yr.$\}$].

This implies that toxic serum concentration would be achieved at lower doses for our population group than for other population groups (*i.e.*, Caucasians), and that they would be more sensitive to changes in their dosage adjustments when concentrations were in or near the therapeutic range.

We ultimately expect that the use of these population pharmacokinetic parameters obtained by our analysis will give the most precise for the determination of an initial maintenance PHT dose and the first dosage adjustment using the Bayesian feedback technique in Japanese patients.

References

1) F. Buchthal, O. Svensmark and P. J. Schiller, *Arch. Neurol.*, **2**, 624 (1960).
2) L. G. Borofsky, S. Louis, H. Kutt and M. Roginsky, *J. Pediatr.*, **81**, 995 (1972).
3) L. Lund, *Arch. Neurol.*, **31**, 289 (1974).
4) H. Kutt and F. McDowall, *J. Am. Med. Assoc.*, **203**, 969 (1968).
5) L. B. Sheiner and S. L. Beal, *J. Pharmacokinet. Biopharm.*, **8**, 553 (1980).
6) T. H. Grasela, L. B. Sheiner, B. Rambeck, H. E. Boenigk, A. Dunlop, P. W. Mullen, J. Wadsworth, A Richens, T. Ishizaki, K. Chiba, H. Miura, K. Minagawa, P. G. Blain, J. C. Mucklow, C. T. Bacon and M. Rawlins, *Clin. Pharmacokinet.*, **8**, 355 (1983).
7) R. Miller, M. Rheeders, C. Klein and I. Suchet, *S. Af. Med. J.*, **72**, 188 (1987).
8) S. L. Beal and L. B. Sheiner, NONMEM Users Guide, (Technical Report), Division of Clinical Pharmacology, University of California, San Francisco, 1979.
9) L. B. Sheiner, B. Rosenberg and V. V. Marathe, *J. Pharmacokinet. Biopharm.*, **5**, 445 (1977).
10) L. B. Sheiner, S. L. Beal, B. Rosenberg and V. V. Marathe, *Clin. Pharmacol. Ther.*, **26**, 90 (1979).
11) E. Yukawa, S. Higuchi and T. Aoyama, *J. Clin. Pharm. Ther.*, **14**, 71 (1989).
12) Y. Kohda, N. Nishihara, S. Isozaki, Y. Saitoh, F. Nakagawa and Z. Tamura, *J. Pharmacobio-Dyn.*, **6**, 46 (1983).
13) Y. Kohda, K. Nishihara, Y. Saitoh, F. Nakagawa and Z. Tamura, *Yakuzaigaku*, **42**, 225 (1982).
14) K. Schmidt-Nielsen, *Fed. Proc.*, **29**, 1524 (1970).
15) A. Richen, *Clin. Pharmacokinet.*, **4**, 153 (1979).