Pharmacokinetic-Pharmacodynamic Modeling of the Anticonvulsant and Electroencephalogram Effects of Phenytoin in Rats

O. E. DELLA PASCHOA, J. W. MANDEMA, R. A. VOSKUYL and M. DANHOF

Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, University of Leiden, University of Leiden, P.O. Box. 9503, 2300 RA Leiden, The Netherlands (O.E.D.P., M.D.), Stanford University School of Medicine, Department of Anesthesia, Palo Alto, California (J.W.M.) and Instituut voor Epilepsiebestrijding, Heemstede, The Netherlands (R.A.V.)

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ABSTRACT

In this study a pharmacokinetic-pharmacodynamic model is proposed for drugs with nonlinear elimination kinetics. We applied such an integrated approach to characterize the pharmacokinetic-pharmacodynamic relationship of phenytoin. In parallel, the anticonvulsant effect and the electroencephalogram (EEG) effect were used to determine the pharmacodynamics. Male Wistar-derived rats received a single intravenous dose of 40 mg kg⁻¹ phenytoin. The increase in the threshold for generalized seizure activity (TGS) was used as the anticonvulsant effect and the increase in the total number of waves in the 11.5 to 30 Hz frequency band was taken as the EEG effect measure. Phenytoin pharmacokinetics was described by a saturation kinetics model with Michaelis-Menten elimination. \( V_{\text{max}} \) and \( K_{\text{m}} \) values were, respectively, 386 ± 31 µg min⁻¹ and 15.4 ± 2.2 µg ml⁻¹ for the anticonvulsant effect in the cortical stimulation model and 272 ± 31 µg min⁻¹ and 5.9 ± 0.7 µg ml⁻¹ for the EEG effect. In both groups, a delay to the onset of the effect was observed relative to plasma concentrations. The relationship between phenytoin plasma concentrations and effect site was estimated by an equilibration kinetics routine, yielding mean \( k_{\text{eq}} \) values of 0.108 and 0.077 min⁻¹ for the anticonvulsant and EEG effects, respectively. The EEG changes in the total number of waves could be fitted by the sigmoid \( E_{\text{max}} \) model, but \( E_{\text{max}} \) values could not be estimated for the nonlinear relationship between concentration and the increase in TGS. An exponential equation \( (E = E_0 + B_0 \cdot C^n) \) derived from the sigmoid \( E_{\text{max}} \) model was applied to describe the concentration-anticonvulsant effect relationship, under the assumption that \( E_{\text{max}} \) values cannot be reached within acceptable electric stimulation levels. This approach yielded a coefficient \( (B) \) of 2.0 ± 0.4 µA ml⁻¹ and an exponent \( (n) \) of 2.7 ± 0.9. The derived \( E_{\text{max}} \) value of 12.5 ± 1.3 µg ml⁻¹ for the EEG effect coincided with the “therapeutic range” in humans.

Phenytoin (diphenylhydantoin) has been used extensively in the treatment of seizure disorders. Although its pharmacokinetics has been well reported both in animals and in humans (Olanow and Finn, 1981; Bauer and Blouin, 1983; Shavit et al., 1984; Jones and Wimbish, 1985; Levine and Chang, 1990) the concentration-anticonvulsant effect profile of phenytoin is not thoroughly understood. Consequently, prediction of the accurate PK-PD relationship for both clinical and preclinical scopes is still a difficult undertaking. This can be attributed largely to the lack of adequate quantitative measures of the effect of AED in vivo (Danhof et al., 1992b). The nonlinear pharmacokinetics of phenytoin represents an additional complicating factor (Theodore, 1992).

To date, the numerous models developed to delineate the anticonvulsant properties of antiepileptic drugs in preclinical investigation, such as the kindling and maximal electroshock models, have limitations which prohibit the assessment of the PK-PD relationship (Rundfeldt et al., 1990; Rundfeldt and Lüschner, 1993; Mulzac and Scott, 1993; Dimmock and Baker, 1994). A major limitation is the impossibility to determine repeatedly the anticonvulsant effect intensity within individual rats.

Furthermore, approaches in which the use of antagonists might fully explain the effect of the agonist are not applicable in vivo because of the mechanism of action of phenytoin. The neuropharmacological and biochemical effects of phenytoin are vast. Some of the reported actions of phenytoin in various systems include: (1) changes in the conductance of ionic channels (Jones and Wimbish, 1985); (2) inhibition of calcium uptake and calcium-dependent protein phosphorylation

ABBREVIATIONS: CSM, cortical stimulation model; \( E_0 \), base-line effect; \( E_{\text{max}} \), maximal effect; \( EC_{50} \), concentration at half maximal effect; EEG, electroencephalogram; \( f_u \), free fraction; \( K_{\text{m}} \), Michaelis constant; PK-PD, pharmacokinetic-pharmacodynamic; TGS, threshold for generalized seizure activity; TLS, threshold for localized seizure activity; \( V_{\text{geo}} \), volume of distribution; \( V_{\text{max}} \), maximum metabolic rate; TNW, total number of waves.

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(Twombly et al., 1988); (3) elevation of membrane potential and changes in the amplitudes of synaptic potentials; (4) suppression of bursting activity; (5) increase in cortical levels of \( \gamma \)-aminobutyric acid (Griffith and Taylor, 1988).

With respect to the pharmacokinetics, it is important to consider that the decay in plasma concentration is not linear. Because the rate at which phenytoin is hydroxylated is dose dependent, elimination can follow both first and zero-order processes, depending on the concentration range. A model with Michaelis-Menten elimination is therefore required to describe the pharmacokinetics of phenytoin (Gibaldi and Perrier, 1982).

In addition, one has to consider that the bulk of PK-PD modeling theory and methodology pertains to linear pharmacokinetics with specific drug-receptor interaction systems (Danhof et al., 1992a, 1993). Mathematical models and methods for such nonlinear systems are much less developed (Ritschel and Hussain, 1984; van Rossum and Burgers, 1984; Gillespie, 1993).

The intent of this report was to develop an integrated approach for the assessment of the time course of the effect of phenytoin by use of the EEG and CSM models. The former is based on the use of quantitative EEG parameters as a measure of the effect on brain electric activity (Mandema and Danhof, 1992). The latter consists of the induction of mild convulsive activity, which is evoked by applying electric pulse trains directly to the cortex. The TLS and TGS can be used as measures of the anticonvulsant effect (Voskuyl et al., 1992). In this way, a realistic estimate of the anticonvulsant effect intensity is obtained (Hoogerkamp et al., 1994).

### Methods

#### Study Design

The study protocol was approved by the Ethical Committee for Animal Experimentation. The anticonvulsant activity and the EEG effect of phenytoin were determined in two groups of rats according to a parallel group design. Two groups of male Wistar-derived rats (Sylvius Laboratory Breeding Facility, Leiden, The Netherlands) (225–300 g) were used throughout the study. The animals were housed individually in plastic cages under constant temperature (22°C) and 12-hr light/dark cycle. Laboratory chow (Standard Labo-

#### Surgical Procedure

Seven chronic cortical EEG electrodes were implanted in the skull of the animals (Mandema and Danhof, 1990) 1 week before the experiments for the measurement of EEG signals (group I). Implan-

#### Drug Dosage, Blood Sampling and Pharmacokinetics

A 40 mg · kg\(^{-1}\) dose of phenytoin was infused intravenously at a rate of 0.1 ml · min\(^{-1}\) for 5 min. Phenytoin sodium (Sigma Chemical Co., St. Louis, MO) was dissolved in water alkalinized with 0.1 N sodium hydroxide. Arterial blood samples (100 or 200 ml) were collected in heparinized tubes before and 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 240 and 300 min after drug administration. Blood samples were then centrifuged for 10 min at 5000 rpm and plasma (50 or 100 \( \mu \)l) separated and stored at \(-30^\circ\)C until analysis.

### EEG Measurements and Cortical Stimulation

#### Group I

The output from bipolar leads was continuously monitored by a Nihon Kohden EEG recorder. During the course of the experiment, animals were kept in motion in a slow-speed rotating drum (10 rph) to control the vigilance level. Base-line activity was monitored for 30 min. After drug administration, EEG recordings were continued for 5 hr. The frontocentral lead on the left hemisphere was subjected to on-line aperiodic analysis for quantification of the effect. The aperiodic analysis algorithm calculates the ampli-

#### Phenytoin Assay

Samples were analyzed by the high-performance liquid chromatography technique essentially in the manner outlined by Ratnaraj et al. (1989). A solution of 1.5 \( \mu \)g mephenytoin (internal standard) in 150 ml acetonitrile was added to a 50- or 100-ml plasma sample. The organic phase was subjected to on-line aperiodic analysis for quantification of the effect. The aperiodic analysis algorithm calculates the ampli-

#### Protein Binding

Residual blood was collected by aorta puncture after completion of the experiments and centrifuged for 10 min at 5000 rpm. Plasma was separated and stored at \(-30^\circ\)C until assay. The protein binding was determined for each individual animal by ultrafiltration at 37°C, with use of the Amicon Micropartition System (Amicon Division, Danvers, MA). Three 0.5-ml aliquots of a plasma sample were spiked with phenytoin to a concentration of 10, 50 and 100 \( \mu \)g · ml\(^{-1}\). Total plasma concentration was measured by the described method in a 50-ml sample of the spiked plasma. Separation of free drug from protein-bound drug was carried out at 37°C by filtration of a 400-ml plasma through a YMT ultrafiltration membrane (Amicon) at 1000 \( x \)
g for 10 min. The ultrafiltrate was then analyzed for free drug concentrations.

Data Analysis

The pharmacokinetics and pharmacodynamics of phenytoin were quantified for each individual rat. First, a two-compartment model with Michaelis-Menten elimination was used to describe the plasma concentration-time profile (Gibaldi and Perrier, 1982):

\[
d{C_1}\over dt = \frac{V_{\text{max}} \cdot C_1}{K_m + C_1} + \frac{R}{V_1} - k_{12} \cdot C_1 + k_{21} \cdot C_2
\]

\[
d{C_2}\over dt = k_{12} \cdot C_1 - k_{21} \cdot C_2
\]

with the error model

\[
\log(C_m) = \log(C_{\text{pi}}) + \epsilon_i
\]

where \(dC_1/dt\) is the rate of decline of drug concentration at time \(t\), \(V_1\) the distribution volume, \(V_{\text{max}}\) the theoretical maximum rate of the process, \(K_m\) the Michaelis-Menten constant and \(k_{ij}\) the transfer rate constant from compartment \(i\) to \(j\); 1 and 2 refer to the first and second compartments, respectively. \(C_{\text{mi}}\) is the \(i\)th measured concentration and \(C_{\text{pi}}\) is the predicted concentration according to the PK model. The model was programmed into SIMULINK (The MathWorks Inc., Natick, MA) and a fifth-order Runge-Kutta method was used for integration. The data were fitted to the model by use of the nonlinear least squares routine fmins in MATLAB (The MathWorks Inc., Natick, MA), which uses the simplex method for the minimization. The log-transform is approximately equivalent to a constant CV model.

The hysteresis was collapsed with a hysteresis minimization routine written for MATLAB based on the COLAPS program described previously (Veng-Pedersen et al., 1991). \(C_e\) was calculated by SIMULINK according to the following link model (see fig. 1):

\[
C_e = k_{e0} \cdot e^{k_e t} \cdot C_p
\]

where \(^*C_p\) indicates convolution of the concentration in the central compartment.

The sigmoid \(E_{\text{max}}\) model was used to describe the relationship between drug concentration and EEG effect (Holford and Sheiner, 1982):

\[
E(C_e) = E_0 + \frac{E_{\text{max}} \cdot C^n}{EC_{50} + C^n}
\]

Assuming that \(E_{\text{max}}/EC_{50} = B^n\), the equation can be rewritten as:

\[
E(C_e) = E_0 + B^n \cdot C^n
\]

where \(E(C_e)\) is the observed effect at concentration \(C\), \(E_0\) is the base-line effect value, \(E_{\text{max}}\) is the maximal effect, \(EC_{50}\) is the concentration at half-maximal effect and \(n\) is a constant expressing the shape of the concentration-effect relationship.

Finally, based on the sigmoid \(E_{\text{max}}\) model we have derived an equation to describe the exponential profile of the anticonvulsant effect. This was performed under the assumption that both \(EC_{50}\) and \(E_{\text{max}}\) values are very high and cannot be determined experimentally, without lesion of the brain or animal harm. When drug concentrations are small in relation to \(EC_{50}\), the equation tends to a constant value in the denominator. In practice, the combined parameter \(E_{\text{max}}/EC_{50} = B^n\) may be used to describe drug effect in the range observed:

\[
E(C_e) = E_0 + E_{\text{max}} \cdot C^n
\]

where \(C_e\) is calculated.

**Fig. 1.** Schematic representation of the fitting model used for kinetics and hysteresis minimization.

**Fig. 2.** Time course of phenytoin plasma concentration (\(n \geq 7\)) after i.v. infusion of 40 mg \(\cdot\) kg\(^{-1}\) phenytoin. The markers denote the mean \(\pm\) S.E.. The solid line represents the fit to all individual data. The bars indicate the duration of infusion.
where $E(C_0)$ is the observed effect at concentration $C$, $E_0$ is the baseline effect value, $B$ represents the quotient between $E_{\text{max}}$ and $EC_{50}$, and $n$ is a constant expressing the steepness of the curve.

In both cases the pharmacokinetic model was used to generate drug concentrations at the times of effect measurement. The equations were fitted to the data by the nonlinear least squares regression routine in MATLAB. After applying the Bartlett’s test for non-homogeneity of variances, statistical analysis was carried out by a one-way analysis of variance or the nonparametric Kruskal-Wallis test.

### Results

#### Pharmacokinetics

The kinetic disposition of phenytoin followed a two-compartment model with Michaelis-Menten elimination. The averaged concentration profile is plotted against time in figure 2. $V_{\text{max}}$ and $K_m$ presented somewhat higher values in group II (table 1). Because protein binding was similar in both groups, the lower values of volume of distribution apparently compensated for such differences, without additional consequences for biophase equilibration and pharmacodynamics.

#### Hysteresis

The effect onset was characterized by a temporal delay relative to plasma concentrations in both groups. As shown in figure 3, the hysteresis was minimized successfully by the link model. $k_0$ values of the same magnitude were obtained for the EEG and anticonvulsant effects.

#### Pharmacodynamics

**Group I.** Intravenous administration of phenytoin induced changes in both amplitudes and TNW in the beta frequency band (12.0–30.0 Hz) of the EEG spectral power. The increase in TNW was selected as the EEG effect measure (fig. 4). Because an $E_{\text{max}}$ value was reached, the concentration-EEG effect relationship could be characterized by the sigmoid $E_{\text{max}}$ model (fig. 5).

**Group II.** The anticonvulsant effect of phenytoin was reflected by the elevation of the TGS without any significant alteration in the TLS. As depicted in figure 4, the effect-time course profile followed a pattern remarkably similar to the changes observed in the EEG. However, fitting these data to the sigmoid $E_{\text{max}}$ model was not possible. It seems that $E_{\text{max}}$ cannot be reached at the administered dose. The exponential equation 7 was used to describe the nonlinear profile of the anticonvulsant effect (fig. 5). The pharmacokinetic and pharmacodynamic parameters generated from the PK-PD modeling are summarized in table 1.

### Discussion

In this study we demonstrate the application of an integrated PK-PD approach to characterize the biophase equilibration kinetics and the concentration-effect relationship of phenytoin both in the EEG and in the cortical stimulation models.

The nonlinear pharmacokinetic profile of phenytoin, described by a two-compartment model with Michaelis-Menten elimination, presented high variability. Our data agree with published data, however (Jones and Wimbish, 1985; Itoh et al., 1988). $K_m$ (Michaelis constant) and $V_{\text{max}}$ (maximum metabolism speed) in group I were 5.9 ± 0.7 mg/ml and 272 ± 31 μg · min⁻¹, respectively. In group II, these values were 15.4 ± 2.2 mg/ml and 386 ± 31 μg · min⁻¹, respectively. The statistical significance was determined by ANOVA. In both cases the pharmacokinetic model was used to generate plasma concentrations at the times of effect measurement. The equations were fitted to the data by the nonlinear least squares regression routine in MATLAB. After applying the Bartlett’s test for non-homogeneity of variances, statistical analysis was carried out by a one-way analysis of variance or the nonparametric Kruskal-Wallis test.
abiotic rate) estimates in different populations range from 1.2 to 24.2 μg·ml⁻¹ and 108 to 568 μg·min⁻¹, respectively (Rambeck et al., 1979). The nonlinear phase of decay in plasma concentration (concentrations greater than the Kₘ values) persisted up to 2 hr after administration in both groups (fig. 2). Because the effect predominated over the same time span, modeling of the concentration-effect profile occurred essentially in the nonlinear phase. Plasma protein binding presented only minor variations within the concentration range investigated. The free fraction ranged from 25 to 30%, which is consistent with data published previously (Levine and Chang, 1990).

To exert its anticonvulsant effect, phenytoin needs to reach its site of action in the central nervous system. Phenytoin in the unbound form distributes into transcellular fluids and is present in various tissue compartments, including liver, fat, muscle and brain (Jones and Wimbish, 1985). In principle, one could expect peak brain concentrations after i.v. administration to be reached immediately, because of the high lipophilicity of phenytoin and the relatively high blood flow to the brain. However, previous studies reported that maximum concentrations in brain were observed 6 min after a 2-min i.v. infusion was completed (Ramsay et al., 1979). Such a difference coincides with the delay to the onset of effect and to maximal effect observed in both models investigated.

The temporal delay (hysteresis) between plasma concentrations and effect appears to be a characteristic of PK-PD modeling of several drugs active in the central nervous system (Mandema et al., 1991; Danhof et al., 1992a). The current study does not permit us to establish whether distribution is the major determinant of the observed hysteresis. However, the rationale for this delay is easily understood if one assumes that the resulting pharmacological effect or response is preceded by drug distribution to the site of action. Thus far, there is no consistent report demonstrating the role of other factors in the biophase equilibration of phenytoin which are

**Fig. 4.** Time course of averaged EEG (○) and anticonvulsant (ΔTGS, ▲) effects (n = 7) after i.v. administration of phenytoin (40 mg·kg⁻¹). Plots show changes relative to base-line values and the markers indicate the mean ± S.E. The TLS (▼) was not affected by drug administration. The solid bars represent the duration of infusion.

**Fig. 5.** PK-PD relationships of phenytoin for individual rats in the EEG and CSM. ○, Relationship between phenytoin concentrations and the EEG effect measure derived after i.v. administration. The solid line represents the best fit of the actual data to the sigmoid Eₘₕ model. ▲, Relationship between phenytoin concentrations and the anticonvulsant effect. The solid line represents the best fit to the exponential equation.
known to cause hysteresis, such as coupling mechanisms and effectuation process that follow the drug-receptor interaction. The hysteresis can be modeled mostly by the effect-compartment approach, which postulates the existence of a hypothetical effect compartment linked to the plasma site by a first-order process ($k_{ao}$). Our approach is based on the assumption that distribution kinetics between plasma and effect site is linear and that the same effect-site concentration always evokes the same response, independent of time. This assumption may not hold when interactive metabolites are formed or when there is development of acute tolerance. However, it has been demonstrated that main metabolites of phenytoin have little or no antiepileptic activity (Jones and Wimbish, 1985). Moreover, there is no evidence of the development of tolerance toward the EEG or anticonvulsant effects of phenytoin in these models.

The successful minimization of hysteresis under the assumption of an effect compartment indicates that plasma (central compartment) concentration can reflect the biophase concentration properly (fig. 3). Furthermore, the resulting $k_{ao}$ values for the EEG and anticonvulsant effects do not differ significantly, one may suggest that the biophase for both effects is pharmacokinetically indistinguishable. In fact, this can be correlated with anatopophysiological findings. The anatomical substrate (somatosensory cortex) for assessment of both effects is the same. Furthermore, studies on the brain regional distributions of specific $[^3]$Hphenytoin binding have demonstrated that the cortex is a major binding site (Wong and Teo, 1988).

Regarding the pharmacodynamics, the assessment of the anticonvulsant action is a complex issue. Therefore, useful, accurate measures of the anticonvulsant effect intensity for PK-PD studies are scarce, despite the numerous animal models of epilepsy. Quantitative pharmaco-EEG on the basis of aperiodic analysis has fulfilled most of the requirements for PK-PD modeling. In previous studies it has been successfully used to describe the effect of benzodiazepines (Mandema et al., 1992; Danhof and Mandema, 1992). Application of this technique to antiepileptic drugs has not been reported, however. For benzodiazepines a clear correlation has been established between the EEG effect and the anticonvulsant effect in the pentylentetrazole model (Mandema et al., 1991). Whether this is also the case for phenytoin remains to be determined. To date, it is well known that phenytoin increases levels of $\gamma$-aminobutyric acid in rat cerebral cortex and enhances its uptake (Wong and Teo, 1988). In addition, phenytoin was recently shown to potentiate the increase in the amplitude of cortical high frequency (20–30 Hz) background activity induced by N-methyl-d-aspartate antagonists. This EEG response is considered to be caused by the antagonistic effect exerted by phenytoin on the release of glutamate or on the N-methyl-d-aspartate receptor-linked channels (Popoli et al., 1994). The existing data do not permit the conclusion that the observed EEG effects are relevant to the anticonvulsant action of phenytoin. Further investigation is still required to establish a correlation between the EEG effect and the pharmacological mechanisms involved. Notwithstanding, the primary merit of such a parameter is to allow PK-PD modeling of the effect according to the sigmoid $E_{max}$ model, providing meaningful pharmacodynamic estimates, i.e., $E_{max}$ and $EC_{50}$. Moreover, it is worth noting that in vivo PK-PD modeling of a drug with nonlinear kinetics by the sigmoid $E_{max}$ model has not been reported thus far.

In contrast to the EEG response, the pharmacodynamic endpoint in the CSM is a direct measure of the anticonvulsant action of phenytoin. Particularly important is the fact that the CSM is one of the few models that allow a graded, reproducible and clinically relevant measure of the antiepileptic effect in an individual animal (Hoogerkamp et al., 1994). In addition, the pharmacological meaning of each threshold has been investigated in previous studies (Voskuyl et al., 1992). Analogous to the difference in efficacy in the pentylentetrazole and maximal electroshock screening models, the threshold for localized seizure activity has been shown to reflect the capacity of a drug to block the onset or triggering of seizure activity but the threshold for generalized seizure activity to encompass the capacity of a drug to prevent propagation of the seizure activity. Thus, the elevation of the TGS without alteration in the TLS agrees with the postulated mechanisms of action of phenytoin, which involves inhibition of the propagation of seizure activity.

The PK-PD relationship presented a nonlinear pattern with a steep increase of the effect at the concentration range of 15 to 25 $\mu$g · ml$^{-1}$ (fig. 5). As in previous studies $E_{max}$ could not be measured. This seems to be a common feature of the method for all the antiepileptics (Hoogerkamp et al., 1994). Such a phenomenon might be explained by the impossibility of more frequent measurement of the effect at the early distribution phase. Furthermore, because the current that was applied to the cortex does not discriminate between inhibitory or excitatory systems, another hypothesis to consider is the triggering and overlapping of different mechanisms which distort the actual effect profile at higher current intensity. This has been partially investigated in a comparison between the concentration-anticonvulsant effect profiles of antiepileptic drugs in the pentylentetrazole model and in the CSM. The results indicated that the mechanisms by which seizure activity is evoked in these models are responsible for the differences in effect pattern (Dingemans et al., 1990). This does not diminish the quality and accuracy of the information obtained from the thresholds, however. In terms of modeling, such a nonlinear profile can be fitted by either an exponential function, as the one we have applied here, or by the log-linear model, which is a simplification of the sigmoid $E_{max}$ model. However, the log-linear model does not explain the effect in the absence of the drug and requires the assumption of a threshold for concentrations to produce a significant effect (Holford and Sheiner, 1981).

In fact, the parameters derived from this approach seem to provide a realistic insight into the anticonvulsant effect of phenytoin. Despite the practical constraints mentioned above, both $EC_{50}$ values in the EEG model and the concentration range of effective anticonvulsant action in the CSM are of the same magnitude as the therapeutic levels in humans. Correcting for protein binding, the EEG effect occurred in the range of 1.8 to 6.0 $\mu$g · ml$^{-1}$ whereas a significant elevation of the TGS was observed between 1.75 and 10 $\mu$g · ml$^{-1}$. This is consistent with plasma concentrations yielding effective therapeutic responses in humans (Wolf, 1993; Yoshida et al., 1993).

In conclusion, our results show that the use of concomitant PK-PD modeling provided independent, accurate information about the transport of phenytoin to the site of action,
understanding about the nature of the observed effects and the underlying concentration-effect relationship. Application of this methodology represents an important step forward in the comprehension of the pharmacodynamics of drugs with nonlinear pharmacokinetics.

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References


Send reprint requests to: Prof. Dr. M. Danhof, Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands.