British Journal of Pharmacology (1998) 125, 1610–1616
http://www.stockton-press.co.uk/bjp

Modelling of the pharmacodynamic interaction between phenytoin and sodium valproate

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1 Treatment of epilepsy with a combination of antiepileptic drugs remains the therapeutic choice when monotherapy fails. In this study, we apply pharmacokinetic-pharmacodynamic modelling to characterize the interaction between phenytoin (PHT) and sodium valproate (VPA).

2 Male Wistar rats received a 40 mg kg^{-1} intravenous dose of PHT over 5 min either alone or in combination with an infusion of VPA resulting in a steady-state concentration of 115.5 ± 4.9 $\mu g\ ml^{-1}$. A control group received only the infusion of VPA. The increase in the threshold for generalized seizure activity (ΔTGS) was used as measure of the anticonvulsant effect.

3 PHT pharmacokinetics was described by a pharmacokinetic model with Michaelis-Menten elimination. The concentration-time course and plasma protein binding of PHT were not altered by VPA. The pharmacokinetic parameters V_{max} and K_m were, respectively, 294 ± 63 $\mu g\ min^{-1}$ and 7.8 ± 2.4 $\mu g\ ml^{-1}$ in the absence of VPA and 562 ± 40 $\mu g\ min^{-1}$ and 15.6 ± 0.9 $\mu g\ ml^{-1}$ upon administration in combination with VPA.

4 A delay of the onset of the effect relative to plasma concentrations of PHT was observed. The assessment of PHT concentrations at the effect site was based on the effect-compartment model, yielding mean k_{e0} values of 0.128 and 0.107 min^{-1} in the presence and absence of VPA, respectively.

5 A nonlinear relationship between effect-site concentration and the increase in the TGS was observed. The concentration that causes an increase of 50% in the baseline TGS ($EC_{50\%TGS}$) was used to compare drug potency. A shift of $EC_{50\%TGS}$ from 13.27 ± 3.55 to 4.32 ± 0.52 $\mu g\ ml^{-1}$ was observed upon combination with VPA ($P < 0.01$).

6 It is concluded that there is a synergistic pharmacodynamic interaction between PHT and VPA *in vivo*.

Keywords: Phenytoin; sodium valproate; drug interaction; cortical stimulation; PK/PD modelling

Introduction

The treatment of convulsive disorders with a combination of drugs is often recommended when monotherapy is not effective. The arguments for rational polytherapy rest on the assumption that pharmacodynamic interactions exist when drugs are administered in combination (Reife, 1998). In theory, pharmacodynamic interactions can be supra-additive (synergistic), antagonistic or originate new effects (Graves, 1993). Among drug combinations, the interaction between phenytoin (PHT) and sodium valproate (VPA) can be transient and unpredictable (Levy & Bajpai, 1995; Levy & Koch, 1982; Patsalos & Duncan, 1993). Several studies have been conducted to characterize the pharmacokinetic interaction between PHT and VPA (Brodie, 1992; Rambeck *et al*., 1996; Riva *et al*., 1996). These studies have shown that the pharmacokinetic interaction is the result of changes in the degree of plasma protein binding of PHT, as well as inhibition of its elimination. However, relatively little is known about the pharmacodynamic interaction between the two drugs (Brown & Shokry, 1997; Patsalos & Duncan, 1993; Pisani *et al*., 1990; Wilder & Homan, 1996). The lack of knowledge on the pharmacodynamic interactions between antiepileptic drugs is largely due to difficulties in the assessment of the anticonvulsant effect intensity *in vivo* (Danhof *et al*, 1992). In most animal models, only a single effect measurement is possible within an individual animal. Furthermore, pharmacokinetic changes cannot be identified and separated from pharmacodynamic changes.

The objective of the present study was to assess the pharmacodynamic interaction between VPA and PHT using integrated pharmacokinetic-pharmacodynamic modelling. Thereby, we could determine the acute effect of VPA on the pharmacokinetics and pharmacodynamics of PHT. The cortical stimulation model (Voskuyl *et al*, 1989) was used to measure the anticonvulsant effect intensity of PHT at several time points following its administration either alone or in combination with VPA. In brief, the method consists of controlled induction of mild convulsive activity, which is evoked by applying electric pulse trains of slowly increasing intensity directly to the frontal cortex. The thresholds for localized (TLS) and generalized (TGS) seizure activity can be used as measures of the anticonvulsant effect (Voskuyl *et al*., 1992). Previous results show that 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 of the University of Leiden. Male adult Wistar rats (10–12 weeks old) weighing 200–250 g were used (Harlan C.P.B., Zeist, The Netherlands) throughout the

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study. The rats were housed individually in Perspex cages at constant temperature (21°C) and 12:12 h light-dark cycle. Laboratory chow and water were supplied *ad libitum* except during actual testing. Three groups of eight rats each were used according to a parallel study design. Group I (VPA) received VPA as a continuous intravenous infusion (100 mg kg^{-1} h^{-1}). Steady-state concentrations were rapidly achieved by administering a short bolus of VPA (80 mg kg^{-1}) at time t = 0. VPA concentrations (around 150 μg ml^{-1}) were such to result in no anticonvulsant effect on its own. Group I also received an intravenous bolus injection of saline (as control for the PHT bolus administered to the other groups). Group II (PHT) received a bolus injection of PHT (40 mg kg^{-1}, infusion rate = 0.1 ml min^{-1}) over 5 min in combination with a continuous infusion of saline. The latter was administered as control for the VPA infusion in Group I. Group III (VPA + PHT) was administered as the drug combination, namely, the continuous infusion of VPA and a bolus injection of PHT. The study design is schematically depicted in Figure 1.

Surgery

Surgical procedures were carried out in animals anaesthetized by injection of 1.0 ml kg^{-1} of Hypnorm® (10 mg ml^{-1} fluanisone + 0.315 mg ml^{-1} fentanyl citrate) and 1.0 ml kg^{-1} Dormicum® (5 mg ml^{-1} midazolam). Two stimulation electrodes were implanted bilaterally into the skull of the rats over the motor area of the fronto-parietal cortex as described previously (Voskuyl *et al*., 1989). Cannulae were implanted in the jugular vein and femoral artery for blood sampling and drug administration, respectively. A dual lumen cannula was used for simultaneous administration of the VPA and PHT. Arterial rather than venous blood was sampled to circumvent the effect of metabolism and other haemodynamic factors that affect pharmacokinetic and pharmacodynamic estimates (Gumbleton *et al*., 1994; Tuk *et al*., 1997, 1998).

Drug administration, blood sampling and pharmacokinetics

PHT was administered as a single bolus 1 h after the start of the infusion of VPA (Sanofi, Manchester, U.K.). Phenytoin

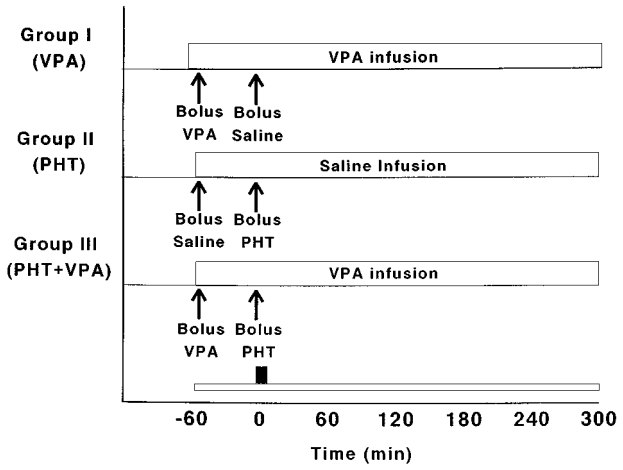

Figure 1 Experimental design used to characterize the pharmacodynamic drug interaction. Both the pharmacokinetics and pharmacodynamics of PHT (i.v. bolus, 40 mg kg^{-1}) were assessed after administration of PHT alone and in combination with a continuous infusion of VPA. The concentration of VPA was maintained at levels that produced no effect on its own in this model (ca. 150 μg ml^{-1}). Group I was used as control for the pharmacodynamics of VPA.

sodium (Sigma, Amsterdam, The Netherlands) was dissolved in water alkalinized with 0.1 N sodium hydroxide. To determine the pharmacokinetics of VPA and PHT, blood samples (100 μl) were collected before and at 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240 and 300 min after drug administration. In order to control haemodynamic factors, blood loss was compensated by injection of an equivalent volume of saline. Plasma levels of PHT were measured by a HPLC technique slightly modified from Lolin *et al*. (1994). Within-day precision was 1.7% for a 10 μg ml^{-1} control sample ($n = 8$). Limit of detection was 0.25 mg ml^{-1} and the assay was linear in the range from 1–100 μg ml^{-1}. VPA concentrations were determined by a gas chromatographic method (Durozard & Bavarel, 1987). Within-day precision was 0.9% for a 470 μg ml^{-1} control sample ($n = 10$). Limit of detection was 1.4 μg ml^{-1} and linearity ranged from 1.4–500 μg ml^{-1}. The validation of both assays was carried out with rat plasma samples previously spiked with either 150 μg ml^{-1} VPA or 25 mg ml^{-1} PHT, respectively.

Plasma protein binding

The plasma protein binding of PHT was determined by ultrafiltration, using the Amicon Micropartition System (Amicon Division, Danvers, MA, U.S.A.) (Mandema *et al*., 1991a). Residual blood was collected by aorta puncture after completion of the experiments and centrifuged at 5000 r.p.m. for 10 min. Plasma were separated and stored at −30°C until assay. The protein binding was determined over a wide PHT concentration range (5–100 μg ml^{-1}) for each individual animal, in the presence and absence of circa 150 μg ml^{-1} VPA. Separation of free drug from protein-bound drug was carried out by filtration of 400 μl plasma through a YMT ultrafiltration membrane (Amicon) at 1090 × *g* for 10 min. The ultrafiltrate was then analysed for free drug concentrations.

Cortical stimulation

The thresholds for localized (TLS) and generalized (TGS) seizure activity were determined during a single test in the same animal, as previously described (Voskuyl *et al*., 1989). Briefly, the electric stimulation consisted of a ramp-shaped bipolar pulse train increasing linearly in intensity (50 pulse sec^{-1}, 0–2400 μA in 20 s, maximal current set at 3000 μA). Stabilization of the baseline threshold was obtained by stimulating the animals twice daily for 2 weeks before the actual experiments. The TLS and TGS were defined as the minimal current intensity necessary to induce clonic movements of the forelimbs and generalized clonic activity, respectively. Baseline thresholds were determined before and after starting the infusion of VPA (groups I and III) or saline (group II). Seizure thresholds were assessed after 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270 and 300 min. Following the bolus injection of PHT (groups II and III) or saline (group I), seizure thresholds were determined. The effect was determined off-line using a video-recording device. The elevation of the thresholds above their baseline represents the anticonvulsant effect.

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 time course of PHT concentrations in plasma (Gibaldi & Perrier, 1982):

$$\frac{dC_1}{dt} = -\frac{V_{max} \cdot C_1}{K_m + C_1} + \frac{R}{V_1} - k_{12} \cdot C_1 + k_{21} \cdot C_2 \quad (1)$$

$$\frac{dC_2}{dt} = k_{12} \cdot C_1 - k_{21} \cdot C_2 \quad (2)$$

with the error model

$$\log C_{mi}) = \log(C_{pi}) + \epsilon_i \quad (3)$$

where dC_1/dt is the rate of decline of drug concentration at time t, V_1 the distribution volume, V_{max} the theoretical maximum rate of the process, K_m the Michaelis-Menten constant and k_{12} and k_{21} are the transfer rate constants from and to the first and second compartments, respectively. C_{mi} is the i-th measured concentration and C_{pi} is the predicted concentration according to the PK model (Della Paschoa *et al*, 1998b).

The delay observed between plasma concentration and onset of the anticonvulsant effect was estimated under the assumption of an effect compartment (Mandema *et al*., 1991b; Veng-Pedersen *et al*., 1991). The concentration at the effect-site (C_e) was calculated according to the link model:

$$C_e = k_{eo} \cdot e^{-k_{eo}\, t} * \; C_p \quad (4)$$

where $*C_p$ indicates convolution of the concentration in the central compartment.

We have derived an equation from the sigmoid E_{max} model to fit the nonlinear profile of the relationship between drug concentration and anticonvulsant effect. This was performed under the assumption that EC_{50} and E_{max} values are both high and cannot be determined experimentally without lesion of the brain or in other ways without harming the animal (Della Paschoa *et al*., 1998b):

$$E(C_e) = E_0 + B^n \cdot C^n \quad (5)$$

where $E(C_e)$ is the observed effect at concentration C, E_0 is the baseline effect value, B represents the ratio between E_{max} and EC_{50}, and n is a constant expressing the steepness of the curve. In order to compare drug potency in the two groups, the model was parameterized. $EC_{50\%TGS}$ was defined as the concentration that causes a 50% increase in the TGS, as compared to baseline values.

In both cases, the pharmacokinetic model was used to generate drug concentrations at the times of effect measurement. The data were fitted using a nonlinear least-squares regression routine in MATLAB (The Mathworks Inc., U.S.A.). After applying the Bartlett's test for non-homogeneity of variances, statistical analysis included one-way ANOVA or the nonparametric Kruskal-Wallis test.

Results

Pharmacokinetics

Adequate steady-state concentrations of VPA were maintained throughout the experiment (Figure 2). Mean ± s.e.mean plasma concentrations of VPA were 122.7 ± 6.4 μg ml^{-1} for group I (VPA) and 115.5 ± 4.9 μg ml^{-1} for group III (PHT + VPA). The pharmacokinetics of PHT could be described by a two-compartment model with Michaelis-Menten elimination (equations (1) and (2)). The time course of PHT concentrations in plasma was not altered by concurrent administration of VPA (Figure 2). However, the pharmacokinetics parameters V_{max}, K_m, and the distribution volume (V_1) were slightly higher in the presence of VPA (Table 1). These differences seemed to compensate each other, without additional consequences to the overall concentration-time course. Plasma protein binding was linear in the concentration range between 25 and 100 μg ml^{-1}. There was no influence of VPA on the degree of protein binding of PHT.

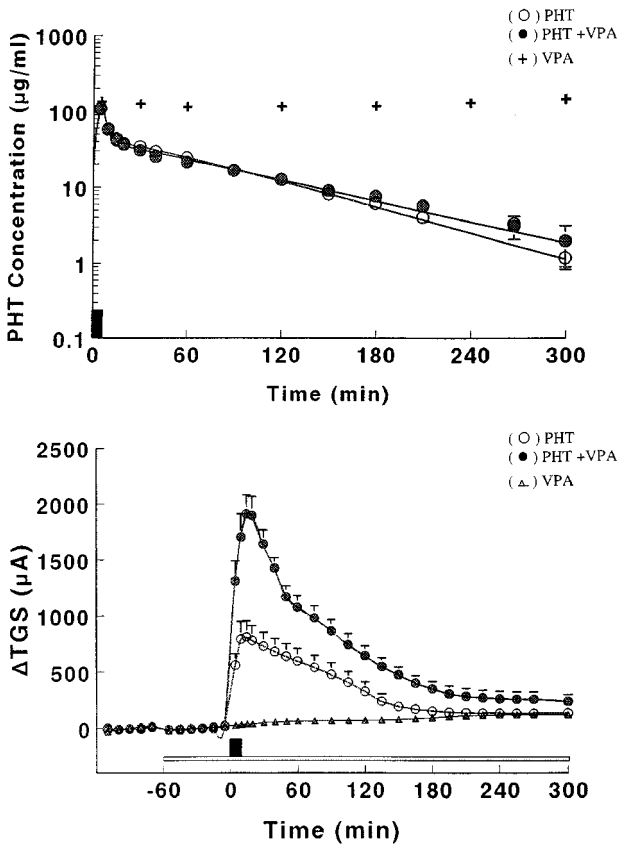

Figure 2 (Upper graph) Time course of PHT plasma concentrations ($n > 7$) following an intravenous infusion of 40 mg kg^{-1} of PHT either in the presence or absence of VPA. The scatters indicate the VPA concentrations during the infusion. The solid bars represent the infusion duration. (Lower graph) Time course of the averaged anticonvulsant effect of PHT alone and in combination with the continuous infusion of VPA. No effect was observed in the control group, which received only the continuous infusion of VPA. Plots show changes relative to baseline value (ΔTGS). The solid bars represent the infusion duration ($n \geqslant 7$).

Pharmacodynamics

Figure 2 also depicts the averaged effect time course before and after drug administration in each individual group. In group I, VPA had no effect on seizure thresholds for localized and generalized seizure activity, as the observed values did not differ significantly from baseline measurements. These results are in agreement with a previous study in which a similar dose of VPA had no effect on its own (Hoogerkamp *et al*., 1994). The effect of PHT was reflected in an elevation of the TGS without alteration of the TLS. The anticonvulsant effect of PHT was considerably enhanced by VPA. The maximum increase in the TGS varied from 864 ± 139 μA, upon administration of PHT only, to 2048 ± 152 μA after administration in combination with VPA.

Biophase equilibration

The onset of the effect was characterized by a temporal delay relative to the plasma concentrations of PHT. The

delay can be easily observed at the end of the infusion of PHT. The maximum increase in the TGS occurs when PHT plasma concentrations are already decaying (Figure 2). Biophase equilibration processes produce a loop (hysteresis) in the relationship between plasma concentrations and effect. This loop explains why plasma concentrations do not correlate directly with the effect (Holford, 1990; Luckow & Della Paschoa, 1997; Mandema *et al*., 1991b). The hysteresis was minimized successfully by the effect-compartment link model (equation (4)). No effect on the biophase equilibration rate constant of PHT was observed. The k_{e0} values were 0.128 ± 0.020 and 0.107 ± 0.021 min^{-1} in the absence and presence of VPA, respectively (Table 1). The values of the rate constant k_{e0} were used to calculate the PHT concentrations at the effect site.

Table 1 Comparison of the pharmacokinetics and pharmacodynamics of PHT after single administration and in combination with a continuous infusion of VPA ($n \geqslant 7$, mean $\pm$ s.e.mean)

Pharmacokinetics	*PHT*	*PHT+VPA*
V_{max} [μg min^{-1}]	294 ± 63	562 ± 40**
K_m [μg ml^{-1}]	7.8 ± 2.4	15.6 ± 0.9*
V_1 [ml]	517 ± 69	631 ± 28*
k_{12} [min^{-1}]	0.3115 ± 0.0346	0.2303 ± 0.0134*
k_{21} [min^{-1}]	0.0719 ± 0.0032	0.0661 ± 0.0025
f_u [%]	21.8 ± 0.9	23.7 ± 0.9
Pharmacodynamics		
K_{e0} [min^{-1}]	0.107 ± 0.021	0.128 ± 0.020
E_0 [μA]	465.3 ± 25.3	469.0 ± 19.3
B [μA ml mg^{-1}]	85.2 ± 44.7	110.1 ± 35.4
n	0.888 ± 0.081	0.902 ± 0.05
$EC_{50\%TGS}$ [μg ml^{-1}]	13.27 ± 3.55	4.32 ± 0.52*
$EC_{50\%TGS}$ unbound [μg ml^{-1}]	2.82 ± 0.71	1.12 ± 0.16*

Statistical significance: *$P<0.05$, **$P<0.01$ (ANOVA).

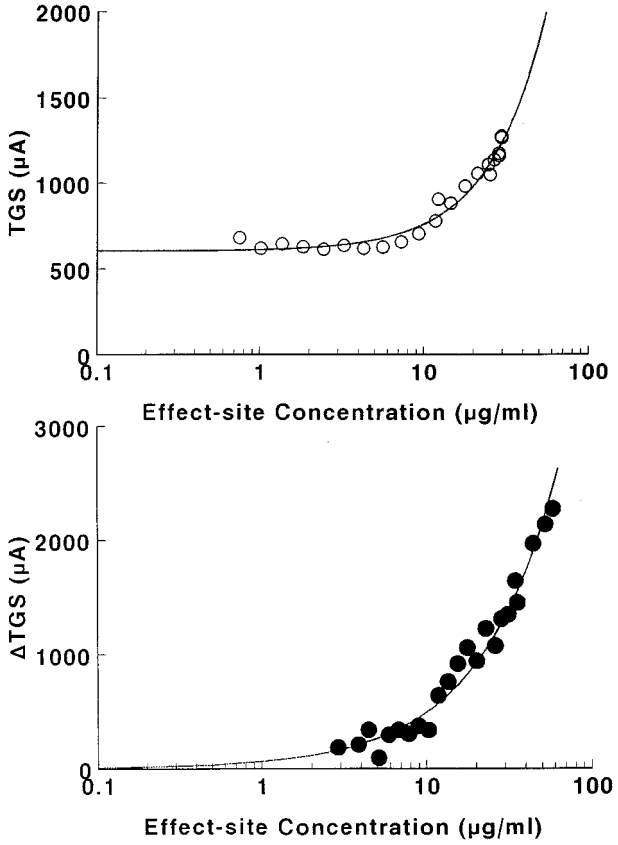

Figure 3 Individual relationship between PHT effect-site concentration and the anticonvulsant effect after intravenous administration (40 mg kg^{-1}) of PHT alone and in combination with a continuous infusion of VPA. The solid line represents the best fit to an exponential equation.

Concentration-effect relationship

In both groups, modelling of the concentration-effect relationship of PHT occurred essentially in the nonlinear pharmacokinetics phase. The exponential equation (6) was used to describe the nonlinear relationship between PHT concentration and the increase in the TGS (Figure 3). In addition to the biophase equilibration delay, pharmacokinetic-pharmacodynamic estimates were calculated taking into account individual differences in the protein binding of PHT. The effect of VPA on the pharmacodynamics of PHT cannot be easily assessed by visual inspection of the concentration-effect curve because of the exponential increase in the TGS. However, $EC_{50\%TGS}$ values reveal that VPA enhances of the anticonvulsant effect of PHT. The unbound $EC_{50\%TGS}$ values of PHT were twofold smaller in the presence of VPA (Figure 4). Both pharmacokinetics and pharmacodynamic estimates generated from pharmacokinetic-pharmacodynamic modelling are summarized in Table 1.

Discussion

In this study, we demonstrate the application of integrated pharmacokinetic-pharmacodynamic modelling to characterize the pharmacodynamic interaction between PHT and VPA. Because drug interactions with PHT usually involve pharmacokinetic changes (Brodie, 1992; Rambeck *et al*., 1996; Riva *et al*., 1996), it has not been possible thus far to characterize quantitatively, the pharmacodynamic interaction between these compounds *in vivo*. In a previous study, we have been able to identify qualitatively the changes in the anticonvulsant effect of PHT induced by VPA. Specific changes in the ictal pattern and seizure duration could be demonstrated using quantitative behaviour analysis (Della Paschoa *et al*., 1998a).

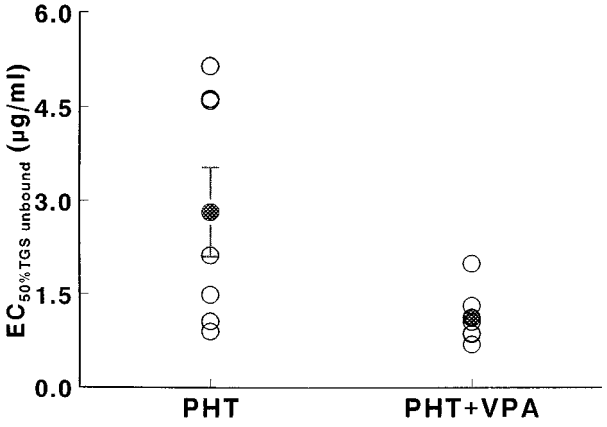

Figure 4 VPA enhances the anticonvulsant effect of PHT. The pharmacodynamic interaction between the two drugs is revealed by a 2 fold decrease in $EC_{50\%TGS}$ values, i.e., the PHT concentration required to induce a 50% increase in the baseline values of the TGS.

The results of the present investigation confirm those observations and show that assessment of the individual concentration-effect relationships is necessary to characterize pharmacodynamic interactions quantitatively.

There was no statistically significant effect of VPA on the concentration time course of PHT. Maximal concentrations of PHT reached at the end of the bolus injection (t = 5 min) were 125.0 ± 7.9 and 116.4 ± 4.6 $\mu g\ ml^{-1}$ for group II and III, respectively. The nonlinear pharmacokinetics of PHT was described by a two-compartment model with Michaelis-Menten elimination. The differences found in some of the pharmacokinetic parameters may be explained by interindividual variability. Large interindividual variability is a known characteristic of saturation pharmacokinetics (Della Paschoa *et al*., 1998b; Dill *et al*., 1956). However, V_{max}, K_m and volume of distribution seem to compensate each other, without affecting total plasma concentrations.

Investigations in man have shown that VPA shares a binding site on serum albumin with PHT and displaces it (May & Rambeck, 1990; Monks & Richens, 1980; Nation *et al*., 1990; Tokola & Neuvonen, 1983). It has been proposed that VPA is also capable of inhibiting the metabolism of PHT (Kutt, 1995). The metabolic inhibition is accompanied by subsequent increase in circulating free levels of PHT upon chronic treatment (Bruni *et al*., 1979, 1980). These observations indicate that a potential pharmacokinetic interaction should be taken into account when studying the pharmacodynamic interaction quantitatively (Della Paschoa *et al*., 1998b).

A clear distinction must be made between the free fraction and the free concentration of PHT. Since only unbound drug is pharmacologically active, changes in free concentrations ought to be considered primarily. Re-equilibration and displacement processes may lead to changes in the value of the free fraction, but the free concentration of PHT may remain unchanged (Cloyd, 1991; Barre *et al*., 1988; Nation *et al*., 1990). In the present study, no influence of VPA on the free concentrations and on the degree of plasma protein binding of PHT was observed. Previous investigations on the correlation between free concentrations of PHT and the dose of VPA have yielded similar findings as in our study (Johnson *et al*., 1989; Scheyer & Mattson, 1991, 1997). Furthermore, unchanged free and total concentrations of PHT suggest that the metabolism of PHT has not been altered by VPA (Scheyer *et al*., 1990).

These observations in animals contrast with data in clinical settings, where it has been shown that PHT protein binding can be altered substantially by VPA (Scheyer & Mattson, 1995; Cramer & Mattson, 1979). Such differences can be explained by the different doses and plasma concentrations of VPA. At high concentrations of VPA, the protein binding of PHT can be altered by VPA, which shares the same binding site on albumin (Gomez Bellver *et al*., 1993; Scheyer & Mattson, 1995). Because of nonlinear pharmacokinetics, drug interactions with PHT may have different implications depending on the concentration range.

With regard to the pharmacodynamics, the increase in TGS is a direct measure of the anticonvulsant effect of PHT (Danhof *et al*., 1992; Hoogerkamp *et al*., 1994). The elevation of the TGS without alteration of the TLS corresponds with the mechanisms of action of PHT (Della Paschoa *et al*., 1998b). However, the elevation of the TGS did not occur simultaneously with the increase in PHT plasma concentrations. In spite of the high lipophilicity of PHT and the relatively high blood flow to the brain, it has been shown that there is a delay to the onset of effect and to maximum effect (Ramsay *et al*., 1979; Ramzan & Levy, 1989; Sechi *et al*., 1989). The hysteresis between plasma concentrations and effect has been minimized under the assumption of an effect-site linked to the central (plasma) compartment by a first-order process (k_{e0}) (Della Paschoa *et al*., 1998b). The values obtained for k_{e0} indicate that the rate of transfer of PHT to the effect-site is not altered by VPA.

The parameters derived from pharmacokinetic-pharmacodynamic modelling provided a realistic insight into the concentration-effect relationship of PHT. The nonlinear relationship between concentration and the anticonvulsant effect showed a steep increase at the concentration range between 10 and 20 $\mu g\ ml^{-1}$. Estimation of the concentration inducing a 50% increase in the TGS ($EC_{50\%TGS}$) allowed comparison of the drug potency in different treatments. This parameter reflects the actual differences in pharmacodynamics. The presence of VPA caused a shift to the left in the concentration-effect relationship and reduced considerably the values of $EC_{50\%TGS}$ both for total and free concentrations of PHT (Figure 4). These findings are in agreement with a previous report of Chez *et al*. (1994), who showed that the interaction between VPA and PHT was supra-additive whereas neurotoxicity was only additive.

Our study does not allow one to draw conclusions about the pharmacological mechanisms involved in the pharmacodynamic interaction between PHT and VPA. However, it is conceivable that the potentiation of the effect of PHT may result from the effect of VPA on voltage-gated sodium channels and sodium conductance (Löscher, 1998; Ragsdale & Avoli, 1998). PHT binds poorly to resting sodium channels. State-dependent drug block is proposed to reflect an allosteric mechanism in which the channel has a modulated drug receptor site with low affinity when the channel is resting and converts to a high affinity when the channel is inactivated (Hosford *et al*., 1997; Nosek, 1981; Segal & Douglas, 1997). Data about differences in the affinity of PHT and VPA are not available in the literature, but one can assume that at low concentrations VPA binds to the receptor site without inducing detectable anticonvulsant effect. At the cellular level, the presence of VPA would cause an increase in the number of inactivated Na^+ channels, without affecting membrane depolarization or repolarization (Ragsdale & Avoli, 1998; Willow *et al*., 1985). The potentiation of anticonvulsant effect can be explained by increased binding of PHT to the high affinity receptor site.

To conclude, the present study shows that integrated pharmacokinetic-pharmacodynamic modelling may be useful to investigate drug interactions. The approach differentiated pharmacokinetic from pharmacodynamic changes and provided evidence about the nature of the interaction between PHT and VPA.

The authors want to thank Dr P. Edelbroek for the analytical assay of sodium valproate in plasma, K.B. Postel-Westra and M.W. Langemeijer for their technical assistance.

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(Received May 5, 1998
Revised September 17, 1998
Accepted September 18, 1998)