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Prediction of the Concentration of Diphenylhydantoin in the Brain Using a Physiological Pharmacokinetic Hybrid Model

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Abstract□A physiological pharmacokinetic hybrid model was developed in order to predict the disposition kinetics of diphenylhydantoin (DPH) in the brain from the plasma concentration data of DPH. The model was constructed under the assumptions of well-stirred, plasma flow-limited and linear tissue disposition kinetics of DPH. DPH was administered intravenously to the rats at a dose of 10 mg/kg together with/without sodium salicylate (SA; 10 mg/kg) and the DPH concentrations in the plasma and brain were determined. Plasma protein binding of DPH was also determined using equilibrium dialysis technique. Then the model was tested for its predictability of DPH concentrations in the brian from the plasma data of DPH. It was found that the predicted values of DPH concentrations in the brian were in fair agreement with the experimental values in the rats of both treatments. The 2-fold increase in the brain concentrations of DPH by SA-coadministration was predicted well from the plasma concentration and plasma free fraction (f_p) data of DPH using the model. Therefore, the hybrid model was concluded to be very useful for the prediction of the concentrations of DPH in the brain from the plasma concentration data. Finally, DPH concentrations in the human brian was calculated using this model from plasma DPH data in the literature, yet the scale-up of this model to the human is not convinced.

Keywords□Physiological pharmacokinetics, hybrid model, diphenylhydantoin (DPH), prediction, brain disposition, sodium salicylate (SA), plasma protein binding.

In drug therapy, it is important to know the concentrations of a drug in the target organ. Pharmacokinetic theories such as compartment theory, statistical moment theory[1,2] and polyexponential decay model[3], in general, can not give any informations on the drug concentrations in the target organ(s). This is because the parameters obtained by these theories such as Vd_{ss}, K_e, K_{12}, K_{21}, CL and $t_{1/2}$ do not have any physiological or anatomical meanings. Moreover, the effects of the pathological changes, species differences and concurrent drugs[4,5] can not be reflected on the drug concentrations in the target organ by these theories.

To overcome these problems, a physiologically based pharmacokinetic model[6] has been developed. Using this model, drug concentrations in the target organ(s) could be predicted directly from the plasma data in some cases. Some anatomical and physiological parameters such as organ blood flow (Q_T) and organ volume (V_T), together with biochemical parameters such as metabolic enzyme activity and protein binding were incorporated into the model.

Based on this physiological model, some investigators developed an animal scale-up technique[7-12] which enabled the prediction of the concentration profiles of some drugs in the human organs from the *in vivo* and *in vitro* animal data. Although the physiological model could predict the drug concentrations in the target organ(s) from the drug concentration data in plasma, it has some shortcomings as follows[13]: 1) inflexibility of the responses for the many parameters involved in the model, 2) the complexity of the equations used, and eventually, 3) time-consuming for the data analysis.

To overcome these shortcomings, a physiologically based hybrid pharmacokinetic model has been developed[13]. In this hybrid model, only the phamacokinetically important organs such as target organs, elmination organs and main distribution organs are taken into considerations based on both the well-stirred model and classical compartment model.

The hybrid model offers considerably convenient way to predict drug profiles in the target organ from the plasma concentration data, since it needs only a few parameters such as V_T, C_T, Q_T, K_p, and f_p etc, the meanings of which will be explained later in the experimental section of this manuscript. It does not

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need overall informations on the drug disposition in all the organs in the body. Changes in the plasma protein binding of a drug, which might happen by the disease state or drug interactions, can be reflected on the disposition kinetics of the drug in the target organ by this hybrid model.

In this study, a physiologically-based hybrid model was developed in order to predict the brain concentrations of diphenylhydantoin (DPH) from the plasma concentration data. DPH (10 mg/kg) was injected intravenously to the rats together with or without 10 mg/kg of sodium salicylate (SA). The blood pool and the brain were selected as the physiological compartments. The suitability of the model was tested by comparing the predicted DPH concentrations in the brain with the experimentally determined ones. Finally, DPH concentration in human brain was simulated by this model using human plasma DPH data in the literature.

EXPERIMENTAL METHODS

Materials

DPH sodium (Samjin Pharm.), DPH (Sigma), sodium salicylate (SA: Kanto), acetonitrile (HPLC grade: B & J), methanol (HPLC grade: Merck), heparin (Choong Wae Pharm.) and tris reagent (Sigma) were used as purchased. All other chemicals were of reagent grade. For instruments, HPLC (Hewlett-Packard: 1090A model), equilibrium dialysis cell (Plexiglass two chambered apparatus: Technilab Ins.), dialysis membrane (36/32 size: Union Carbide) and tissue-mizer (Fisher) were used.

Animal treatment

Male Wistar rats weighing 230 to 260g were used. All rats were normally fed until the operation. Rats were immobilised at supine position on the operation plate under light ether anesthesia, and PE-50 catheters (Intramedic, Clay Adams) were inserted into the fermoral vein and artery. After recovery from the ether anesthesia, rats of one group (DPH only group, n = 18) were received 10 mg/kg of DPH and rats of another group (DPH-SA group, n = 18) were received 10 mg/kg of DPH and 10 mg/kg of SA intravenously. Drugs were dissolved in the mixed solution of normal saline, propylene glycol and ethyl alcohol (50 : 40 : 10). The injection volume was 200μl/250g body weight. Blood samples (200μl) were withdrawn at the specified time. Plasma were seperated and stored at −20°C until analysis. At specified times, three rats were reanesthesized with ether and whole brain was removed after cutting abdominal artery to prevent blood influx into the brain. Blood remained in the brain was removed by rapid washing with the ice-cold saline and blotted dry with paper tissue. The brains were stored also at −20°C until analysis. The sampling times for brain and blood are shown in Table I.

Table I. Plasma and brain tissue sampling schedule

Rat series (n = 3)	Sampling time (min)					
	2	5	10	15	30	60
A	T					
B	P	T				
C	P	P	T			
D	P	P	P	T		
E	P	P	P	P	X	
F	P	P	P	P	P	X

P; plasma sampling, T; brian sampling, X; brain and plasma sampling.

Serum protein binding of DPH

Serum protein binding of DPH was determined by equilibrium dialysis technique. Tenμl of DPH stock solution (200,500,1000 and 2000μg/ml, respectively) was spiked into 1 ml of serum and the serum was dialysed against 1 ml of tris buffer (pH 7.4) through the dialysis membrane at 37°C for 24 hrs. Effects of SA (40-150μg/ml) on the protein binding of DPH was also examined. Concentrations of DPH and SA in the serum and buffer side of the dialysis cell were determined and free fractions of DPH (f_p) were estimated as follows.

$$f_p = C_{buffer}/C_{serum} \quad \text{(Eq.1)}$$

where C_{buffer} and C_{serum} represent DPH concentrations in buffer and serum side respectively.

Drug analysis

To 100μl of the respective plasma, serum and buffer samples, 200μl of acetonitrile (alkalinized to 0.001 N by NaOH) was added and mixed for 30 seconds. After centrifugation at 3000 rpm for 5 min, 50μl aliquots of the supernatant were injected onto the HPLC column. For the assay of DPH in the brain, 500 mg of the tissue was mixed with 1.5 ml of 0.001 N NaOH and homogenized. Then 3 ml of acetonitrile was mixed for 1 min and the mixture was centrifugated at 3000 rpm for 50 min. One hundredμl aliquots of the supernatant were injected on the HPLC column. HPLC was performed according to the reported method[14] after minor modification. The HPLC sys-

tem was consisted of a pump (Hewlett-Packard, model 1090) with a UV detector adjusted at 214 nm for DPH and 290 nm for SA. The mobile phase was a mixture of 0.05 M phosphate buffer (pH 3.0) and acetonitrile (60:40). The stationary phase was ODS chemically bonded to porous gel (particle size 10μm) packed in a 20 cm stainless steel column (0.46 cm *id*). DPH and SA concentrations in the sample were determined using the peak heights.

Development of hybrid model and simulation of the brian DPH concentration in the rat

A physiological hybrid model developed in this study is shown in Fig. 1. This model was developed under the following assumptions; 1) brain tissue is well-stirred, 2) drug distribution in the tissue is limited by the blood flow rate, and 3) tissue to blood concentration ratio (K_p) is independent of drug concentration. Following equations were used to describe the model. The values of parameters used are listed in Table II. Some parameters were cited from the literature[15] and the others were actually measured in this study.

$$V_T \cdot dC_T/dt = Q_T \ (Ae^{-\alpha t} + Be^{-\beta t} - C_T/K_{pu}/f_p) - f_p \cdot CL_{int} \cdot C_T/K_{pu} \quad \text{(Eq.2)}$$

$$dC_T/dt = Q_T \ (C_p - C_T)/(K_{pu} \cdot f_p)/V_T \quad \text{(Eq.3)}$$

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq.4)}$$

$$K_{pu} = C_t/(C_p \cdot f_p) \quad \text{(Eq.5)}$$

Where V_T, C_T, Q_T, K_{pu}, f_p, CL_{int} and t represent organ volume, organ drug concentration, organ plasma flow, ratio of organ concentration to free plasma concentration, drug free fraction in plasma, intrinsic clearance and time, respectively. A, B, α and β represent intercept (A, B) an the slopes (α, β) of the curves fitted to the conventional 2-compartment model. K_{pu} was calculated using NLS programs[16]. V_T and f_p were determined in this study, and Q_T was cited from the literature[17]. $f_p \cdot CL_{int}$ was determined by *Dose*/*AUC* where *Dose* is the administered dose of DPH and *AUC* is the area under the plasma DPH concentration-time curve. Simulation was performed by Runge-Kutta-Gill method[18] and plasma concentration profiles were analyzed by MULTI program[19] after minor modification. The simulated results were evaluated using mean percent predictability (MPP), mean standard error (MSE), and mean squared standardized error (MSSE) (Appendix).

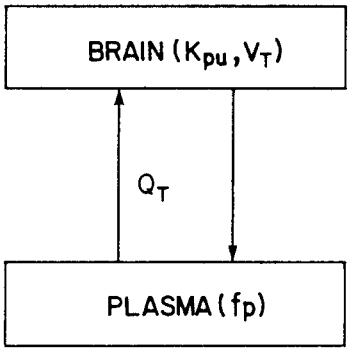

Fig. 1. Diagram of a physiological hybrid model including brain and plasma pool.

K_{pu}, V_T, Q_T and f_p represent free drug partition coefficient between brain and plasma, volume of the brain, plasma flow into the brain and plasma free fraction.

Table II. Parameters used in the simulation

Parameters	Rat (250g)	Man (70 kg)
K_{pu}	6.435	6.435[c]
f_p[a] DPH	0.152 ± 0.015 (n = 18)	0.1[d]
DPH + SA	0.295 ± 0.038 (n = 18)	—
Brain weight (V_T) (g/body)	1.846 ± 0.165 (n = 42)	1500[b]
Plasma flow to brain (Q_T) (ml/min)	1.1[b]	818[b]

[a] Determined by eqilibrium dialysis technique at 37°C. DPH and SA concentration were in the ranges of 2-20 μg/ml and 40-150 μg/ml respectively, [b] cited from reference 17, [c] assumed value, [d] cited from the reference 21.

Prediction of DPH concentration in the human brain

The concentration of DPH in human brain was also simulated from the reported plasma concentration data of the human in the literature[20] using the same model. V_T, Q_T and f_p for human were cited from the literatures[17, 21]. K_{pu} value obtained from the rat was used in the model. Table II summarizes the parameter values used in the simulation.

RESULTS AND DISCUSSION

Serum protein binding

Protein binding of DPH is known to be affected by the concentration of albumin[22-24] and tempera-

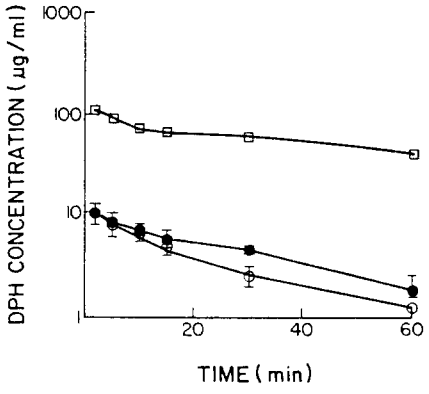

Fig. 2. Observed plasma concentrations of DPH in the DPH only (○) and DPH + SA (●) rats.

SA concentrations in the DPH + SA group were also expressed as □. Each point represents the mean ± S.D. of 3 determinations based on Table I.

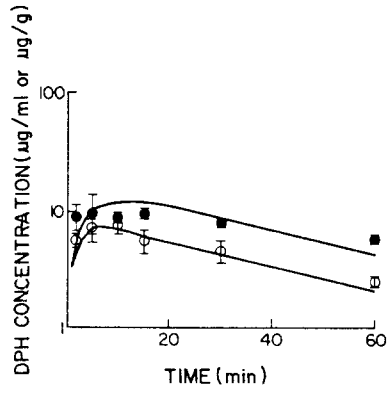

Fig. 3. Observed and simulated brain concentrations of DPH in the DPH only (○) and DPH + SA rats (●).

Each point represents the mean ± S.D. of 3 determinations. Each line represents the simulated brain concentrations of DPH in the DPH only (below) and DPH + SA rats (above), respectively.

ture[25-28]. Protein binding of DPH was determined at 37°C over the actual concentration range of DPH in the plasma after *iv* injection of DPH (10 mg/kg). The free fractions (f_p) of DPH were constant in the concentration range examined and calculated to be 0.152 for the DPH only group and 0.295 for DPH + SA group (Table II). Significant ($p < 10^{-6}$) increase in f_p by *SA* coadministration indicates the replacement of protein binding of DPH by SA.

Plasma and brain concentration profiles

The plasma concentration profiles of DPH and SA in the two groups are shown in Fig. 2. Fig. 3 shows the brain concentration-time curves of DPH in the two groups. SA concentration in the brain was below the detection limit (5μg/ml) in the rats of DPH + SA group. The plasma concentration of DPH was not affected significantly by the coadministration of SA (Fig. 2), however, the brain concentration of DPH was increased 2-folds by SA (Fig. 3). The increased f_p of DPH by the concurrent administration of SA (Table II) may be responsible for the increased diffusion of DPH to the organs such as brain.

Evalulation of the model

The calculated concentration values of DPH in the brain was in good agreement with the experimentally obtained ones in the brain. It was supported by MPP, MSE and MSSE values, which were 96.5 ± 10.5, −0.032 and 0.53 for DPH only group and 103.5 ± 12.5, 0.036 and 0.081 for DPH + SA group (Fig. 3). It means that the model developed in this study predicts fairly well the brain concentration of DPH from the plasma concentration data of DPH.

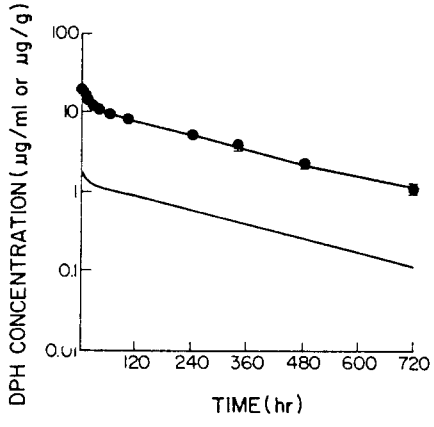

Fig. 4. Brain concentration of DPH in the human simulated (—) from the human plasma concentration data of DPH (●—●) in the literature.

It also implies that all the assumptions adopted for the development of the model are valid.

Prediction of DPH concentration in the human brain using the model

Fig. 4 shows the simulated concentration profile of DPH in the human brain which was calculated using the hybrid model from the plasma concentration data in the literature[20] and parameters listed in Table II. Contary to the rat, the concentration profile of DPH in human brain was calculated to be lower than that in the human plasma. It could be explained by smaller f_p (0.1) and Q_T per V_T (0.545 ml/min/g human brain) for the human than f_p (0.152) and Q_T

per V_T (0.596 ml/min/g human brain) for the rat, which yield much smaller brain to plasma concentration ratio of DPH (K_p) for the human than that for the rat.

Simulation of the concentration profile of DPH in the human brain after intravenous injection was very simple, while the application of the hybrid model to the human should wait further confirmations.

CONCLUSION

A physiologically based pharmacokinetic hybrid model was developed in order to examine the predictability of DPH disposition in the brain from the plasma DPH data. DPH was injected intravenously to the rats together with or without SA, and the plasma and brain concentrations of DPH were determined. SA did not affect the plasma DPH concentrations significantly, but increased the brain DPH concentrations by approximately 2-folds. This may be due to the increased serum free fraction (f_p) of DPH in the presence of SA. In the DPH only rats, the simulated brain DPH concentrations were in good agreement with actual brain concentrations. The simulation was performed using Runge-Kutta-Gill method. The effect of SA on the plasma and brain concentration of DPH was also predictable by incorporating the change of plasma free fraction (f_p) of DPH into the equations of the model. Finally, brain concentrations of DPH in human was estimated using the model from the plasma DPH concentration data in the literature. Prediction of DPH concentration in the human brain would be possible using the hybrid model from the DPH concentration data in the human plasma as long as the assumptions adopted in this study hold. The scale-up of hybrid model from rat to human, however, must wait experimental verification before confirmation.

APPENDIX

Parameters used for the evaluation of the predicatability of the model are as follows;

C_{obs} : Observed concentration.
C_{pre} : Predicted concentration.
PP : Percent predictability.
$PP = (C_{obs}/C_{pre}) \times 100$
SE : Standardized error.
$SE = (C_{obs}-C_{pre})/C_{pre}$
SSE : Squared SE.
$SSE = \sqrt{SE}$
MPP : Mean PP (Σ PP/N).
MSE : Mean SE (Σ SE/N).
$MSSE$: Mean SSE (Σ SSE/N).

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