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Relationships between the Hepatic Intrinsic Clearance or Blood Cell–Plasma Partition Coefficient in the Rabbit and the Lipophilicity of Basic Drugs

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Abstract

The relationships between drug lipophilicity and hepatic intrinsic clearance ($CL_{int,h}$) or red blood cell–plasma partition coefficients (D) have been elucidated for ten highly lipophilic basic drugs with apparent octanol–water partition coefficients at pH 7·4 ($P_{app,oct}$) of 150 or above.

The true octanol-water partition coefficients of the non-ionized drugs (P_{oct}) were used to determine $CL_{int,h}$ and D for the unbound drugs ($CL_{int,h,f}$ and D_f, respectively), and $CL_{int,h,f}$ and D_f for the non-ionized and unbound drugs ($CL_{int,h,fu}$ and D_{fu}, respectively). The total clearance values were determined at steady state by infusion studies of individual drugs in rabbits. There was better correlation between log P_{oct} and log $CL_{int,h,fu}$ ($r = 0.974$) than between log P_{oct} and log $CL_{int,h,f}$ ($r = 0.864$). The D values were calculated from the blood-plasma concentration ratio. There was a better correlation between log P_{oct} and log D_{fu} ($r = 0.944$) than between log P_{oct} and log D_f ($r = 0.612$). The regression equations obtained were $CL_{int,h,fu} = 0.0875 \times P_{oct}^{1.338}$ and $D_{fu} = 0.0108 \times P_{oct}^{0.970}$, respectively.

These results show that the $CL_{int,h}$ and D of highly lipophilic basic drugs can be predicted from P_{oct} by taking f_u into consideration. By applying these parameters to a physiologically based pharmacokinetic model it might be possible to predict the pharmacokinetics of unknown basic drugs.

Although physiologically based pharmacokinetic models can be used to predict drug effects, toxicity and interactions, there are many difficulties associated with determining the pharmacokinetic parameters for a physiologically based pharmacokinetic model in man, and few parameters are available. Therefore, it is useful to predict the pharmacokinetic parameters in man by means of easily obtained drug characteristics. Recently, several studies have been conducted to establish the quantitative relationship between drug structure–activity and pharmacokinetic parameters. Attempts have been made to predict clearance (Kaneniwa et al 1979; Hiura et al 1984) and distribution (Watanabe & Kozaki 1978; Grabowski et al 1980; Greenblatt et al 1983) in man from the relationship between the oil–water partition coefficient (P_{app}) and values of clearance and distribution observed in animal experiments.

We established a physiologically based pharmacokinetic model for pentazocine (Ichimura et al 1984) and biperiden (Nakashima et al 1987) in animals, and predicted the disposition kinetics in man on the basis of an animal scale-up method. To predict the disposition kinetics using the physiologically based pharmacokinetic model it is necessary to determine the tissue–plasma concentration ratio (K_p), the hepatic intrinsic clearance ($CL_{int,h}$), and the red blood cell–plasma partition coefficient (D). We reported good correlation between log K_p for the non-ionized and unbound drug and the log P_{app} value of the non-ionized drug (P) measured in experiments with rabbits (Yokogawa et al 1990).

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The purpose of this study was to determine the regression equations for the relationship between log P and log $CL_{int,h}$ and that between log P and log D, in the rabbit, for ten highly lipophilic basic drugs with apparent partition coefficients of 150 or above in an octanol–water system at pH 7·4.

Materials and Methods

Materials

Biperiden, haloperidol (Dainippon, Osaka, Japan), chlorpromazine, clotiazepam (Yoshitomi, Osaka, Japan), clomipramine (Ciba Geigy, Japan), diazepam (Takeda, Osaka, Japan), nitrazepam, promethazine (Shionogi, Osaka, Japan), trihexyphenidyl (Nippon Lederle, Japan) and pentazocine (Sankyo, Tokyo, Japan) were used as supplied. Other chemicals were of reagent grade and used without purification.

Animal experiments

Adult male albino rabbits, 2.1 ± 0.2 kg (mean ± s.d.), were studied, essentially as described previously (Yokogawa et al 1986). Briefly, under light anaesthesia the femoral artery and vein were cannulated with polyethylene tubing and a urinary catheter was placed in the bladder. To determine total body clearance (CL_{total}) and renal clearance (CL_{urine}), intravenous infusions were performed after an intravenous bolus injection of the priming dose. After 16 h a steady state level of drug plasma concentration was achieved, urine was collected, and blood was withdrawn through the femoral artery at the midpoint of the collection period. The plasma was separated by centrifugation. The plasma and the urine were stored at −30°C until assay.

Determination of blood–plasma concentration ratio (RBP)

A conventional in-vitro method was used. After administration of heparin (1 mL kg^{-1}; 100 units), whole blood was collected via the femoral artery cannula. Samples (0·1 mL) of isotonic buffer solution containing different amounts of each drug were added to whole blood (5 mL). The samples were incubated with slow shaking for 30 min at 37°C. The concentrations of each drug in the plasma after separation by centrifugation and in whole blood were then assayed.

Determination of the unbound fraction of drug in serum (f_p)

The extent of drug binding to rabbit serum protein was measured in each group by the equilibrium dialysis technique with a sample volume of 0·8 mL as described previously (Yokogawa et al 1986).

pK_a and lipophilicity

pK_a values were determined by potentiometric titration at 37°C (Albert & Serjeant 1971). Octanol was used as the organic solvent and isotonic phosphate buffer (pH 7·4) as aqueous solution. An exactly measured amount (3–100 mL) of each solution was transferred to a siliconized glass-stoppered flask and shaken for 16 h at 37°C to achieve complete equilibrium. The aqueous phase was centrifuged at 3000 *g* for 10 min, then extracted into ether and assayed.

Assay for drugs

Drug concentrations in plasma and urine were determined by gas chromatography as described previously (Yokogawa et al 1985) using a GC-7A (Shimadzu, Kyoto, Japan) chromatograph equipped with an FTD-8 (Shimadzu) nitrogen-phosphorus detector and a 25 m × 0·24 mm i.d. flexible fused silica capillary column silanized and coated with a solution of SE-52 (ULBON R HR-52, Sinwa Kako, Japan).

Data analysis

The fit between the observed (Y_{obs}) and predicted (Y_{calc}) values of $CL_{int,h}$ or D for each drug was measured on the basis of the coefficient of determination, r^2, calculated from the equation:

$$r^2 = 1 - \sum dev^2/Sy^2$$

where $Sy^2 = \sum Y_{obs}^2 - (\sum Y_{obs})^2/n$, $dev^2 = (Y_{obs} - Y_{calc})^2$, and n is the number of determinations (Tsuji et al 1985).

Results

Determination of the true octanol–water partition coefficient of the non-ionized drugs (P_{oct})

Table 1 lists the apparent partition coefficients ($P_{app,oct}$) of each drug, calculated according to equation 1:

$$P_{app,oct} = ((A_0 - A) \times P_W)/(P_0 \times A) \quad (1)$$

where A_0, A, P_0 and P_W are the initial drug concentration in the aqueous phase, the equilibrium drug concentration in the aqueous phase, the volume of the octanol phase, and the volume of the aqueous phase, respectively.

The P_{oct} of each drug at pH 7·4 was estimated. According to Henderson–Hasselbach theory the ionized state of a weakly basic drug in the aqueous phase is given by equation 2:

$$X_{wf}/X_{wi} = 10^{(pH - pKa)} = F \quad (2)$$

where X_{wf} and X_{wi} are the amounts of non-ionized drug and ionized drug, respectively. The observed pK_a of each drug is given in Table 1. The amounts of drug in the aqueous and octanol phases are given by equations 3 and 4:

$$X_w = X_{wf} + X_{wi} \quad (3)$$

$$X_{of} = X_0 - X_w \quad (4)$$

where X_w and X_{of} are the amounts of the drug in the aqueous and octanol phases, respectively, and X_0 is the initial amount of the drug. Combining equations 2 and 3 gives equation 5.

$$X_{wf} = X_w \times F(F + 1) \quad (5)$$

P_{oct} for each drug was estimated according to equation 6; the log P_{oct} values are given in Table 1.

$$P_{oct} = (X_0 - X_w)/X_{wf} = (X_0 - X_w) \times (F + 1)/(X_w \times F)$$
$$= P_{app} \times (F + 1)/F \quad (6)$$

The relationship between hepatic intrinsic clearance ($CL_{int,h}$) and P_{oct}

The observed values of f_p, RBP, CL_{total} and CL_{urine} per unit body weight for each drug, determined at steady state in rabbits, are listed in Table 2. The hepatic clearance (CL_h) per unit

Table 1. Physicochemical properties of the ten basic drugs.

Drug	pK_a	Apparent octanol–water partition coefficient	Logarithm of the octanol–water partition coefficient of the non-ionized form of the drug
Pentazocine	8·5	150	3·31
Nitrazepam	3·4	162	2·21
Haloperidol	7·8	485	3·23
Biperiden	8·8	678	4·25
Diazepam	3·5	970	2·99
Promethazine	9·1	1270	4·81
Trihexyphenidyl	8·7	1470	4·49
Chlorpromazine	9·3	1900	5·19
Clotiazepam	3·6	3060	3·49
Clomipramine	8·5	3800	4·71

All values are at pH 7·4 and 37°C.

Table 2. Pharmacokinetic parameters of ten basic drugs at steady state in rabbits.

Drug	Unbound fraction of the drug in serum*	Blood–plasma concentration ratio*	Total clearance (mL min^{-1} kg^{-1})	Renal clearance† (mL min^{-1} kg^{-1})
Pentazocine	0·40 ± 0·013 (15)	1·55 ± 0·06 (14)	42·3 ± 3·3	4·2 ± 1·4
Nitrazepam	0·17 ± 0·007 (10)	0·72 ± 0·03 (10)	9·25 ± 1·2	0·312 ± 0·124
Haloperidol	0·23 ± 0·008 (8)	1·1 ± 0·04 (8)	61·6 ± 8·4	0·74 ± 0·343
Biperiden	0·39 ± 0·04 (11)	1·17 ± 0·05 (6)	78·7 ± 3·8	1·46 ± 0·6
Diazepam	0·091 ± 0·004 (8)	0·873 ± 0·03 (10)	28·9 ± 4·7	0·34 ± 0·155
Promethazine	0·22 ± 0·009 (8)	1·88 ± 0·07 (10)	106·0 ± 11·1	1·13 ± 0·606
Trihexyphenidyl	0·37 ± 0·01 (10)	1·23 ± 0·04 (6)	77·4 ± 6·9	0·862 ± 0·39
Chlorpromazine	0·095 ± 0·003 (10)	1·25 ± 0·03 (9)	79·2 ± 8·4	0·57 ± 0·238
Clotiazepam	0·03 ± 0·001 (10)	0·954 ± 0·02 (10)	29·9 ± 1·6	0·13 ± 0·06
Clomipramine	0·067 ± 0·002 (10)	1·45 ± 0·04 (7)	86·7 ± 8·9	0·267 ± 0·147

Each value represents the mean ± s.e.m. *The number of experiments is given in parentheses. † Each value was obtained from three experiments.

body weight was estimated by subtracting CL_{urine} from CL_{total}. The hepatic intrinsic clearance of the unbound drug ($CL_{int,h,f}$) is given by equation 7:

$$CL_{int,h,f} = (RBP \times Q_{liver} \times CL_h)/((RBP \times Q_{liver} - CL_h) \times f_p) \quad (7)$$

where Q_{liver} is the hepatic blood flow. Taking the fraction of the non-ionized form of the free concentration in extracellular space (f_u) into consideration, the hepatic intrinsic clearance of the unbound and non-ionized drug ($CL_{int,h,fu}$) is represented by equation 8:

$$CL_{int,h,fu} = (RBP \times Q_{liver} \times CL_h)/((RBP \times Q_{liver} - CL_h) \times f_p \times f_u) \quad (8)$$

The $CL_{int,h,f}$ and $CL_{int,h,fu}$ values were estimated by substituting Q_{liver} (68 mL min^{-1} kg^{-1}; Ichimura et al 1985), RBP, CL_h, f_p and f_u into equations 7 and 8. Fig. 1 shows the relationship between these values and P_{oct} as a logarithmic plot.

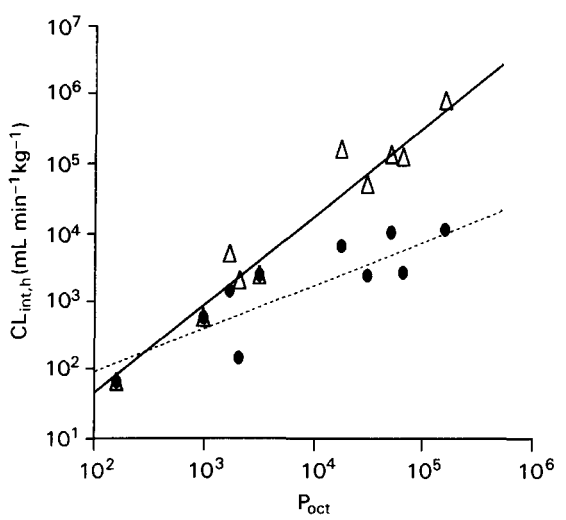

FIG. 1. Relationship for ten basic drugs between the octanol–water partition coefficients of the non-ionized drugs (P_{oct}) and the hepatic intrinsic clearance of the unbound drugs (● $CL_{int,h,f}$; r = 0·864), and that of the non-ionized and unbound drugs (△ $CL_{int,h,fu}$; r = 0·974). The dashed and continuous lines are the regression lines for $CL_{int,h,f}$ and $CL_{int,h,fu}$, respectively.

The regression equations for $CL_{int,h,f}$ and $CL_{int,h,fu}$ are given in equations 9 and 10, respectively.

$$CL_{int,h,f} = 3{\cdot}828 \times P_{oct}^{0{\cdot}676} \quad (9)$$

$$CL_{int,h,fu} = 0{\cdot}0875 \times P_{oct}^{1{\cdot}338} \quad (10)$$

The correlation coefficients were 0·864 and 0·974, respectively.

Relationship between blood cell-plasma partition coefficient (D) and P_{oct}

The RBP of the highly lipophilic drugs is estimated by use of equation 11 assuming that blood cell–plasma partition rapidly reaches equilibrium:

$$RBP = C_b/C_p \quad (11)$$

where C_b and C_p are the concentrations in blood and plasma, respectively. The blood cell drug concentration (C_{rbc}) was estimated by use of equation 12 (Derendorf 1987):

$$C_{rbc} = (C_b - C_p \times (1 - Ht))/Ht \quad (12)$$

where Ht is the hematocrit.

Assuming that only unbound drug penetrates the red blood cells, equation 13 can be obtained from equations 11 and 12:

$$D_f = C_{rbc}/(C_p \times f_p) = (RBP - (1 - Ht))/(f_p \times Ht) \quad (13)$$

where D_f is the blood cell-plasma partition coefficient. The blood cell-plasma partition of the unbound and non-ionized drug (D_{fu}) which divided the D_f by the f_u is represented by equation 14.

$$D_{fu} = C_{rbc}/(C_p \times f_p \times f_u) = (RBP - (1 - Ht))/(f_p \times f_u \times Ht) \quad (14)$$

The D_f and D_{fu} values of each drug were estimated by substituting the observed values of RBP, f_p and f_u and the Ht (0·35) (Yokogawa et al 1986) into equations 13 and 14, respectively. Fig. 2 shows the relationship between these values and P_{oct} as a logarithmic plot. The linear regression equations for D_f and D_{fu} are given in equations 15 and 16, respectively.

$$D_f = 0{\cdot}641 \times P_{oct}^{0{\cdot}287} \quad (15)$$

$$D_{fu} = 0{\cdot}0108 \times P_{oct}^{0{\cdot}970} \quad (16)$$

The correlation coefficients were 0·612 and 0·944, respectively.

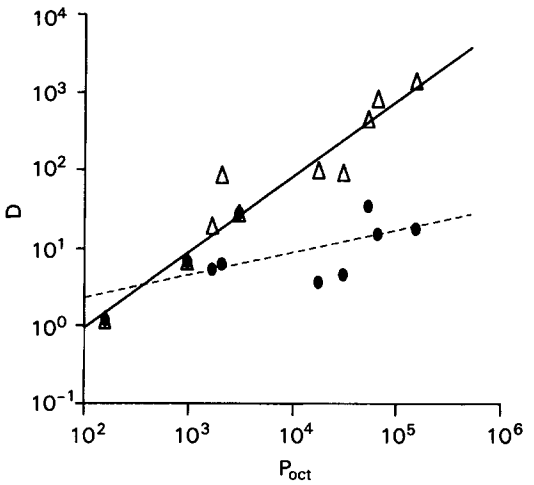

FIG. 2. Relationship for ten basic drugs between the octanol–water partition coefficients of the non-ionized drugs (P_{oct}) and the red blood cell–plasma partition coefficients of the unbound drugs (● D_f; r = 0·612), and those of the non-ionized and unbound drugs (△ D_{fu}; r = 0·944). The dashed and continuous lines are the regression lines for D_f and D_{fu}, respectively.

Contribution of f_u to the prediction of $CL_{int,h}$ and D

If f_u was not taken into consideration, the regression equation obtained was:

$$CL_{int,h,f} = 0{\cdot}248 \times P_{app,oct}^{1{\cdot}289} \quad (r = 0{\cdot}830) \qquad (17)$$

Fig. 3 shows the relationship between the observed values of $CL_{int,h,f}$ and the values calculated from equation 17. The figure also shows the relationship between the observed values of $CL_{int,h,f}$ and the calculated values of $CL_{int,h,fu}$ estimated from equation 10 multiplied by f_u. Taking f_u into consideration, the correlation coefficients increased from 0·532 to 0·837.

Not taking f_u into consideration, the regression equation obtained for D_f was:

$$D_f = 0{\cdot}102 \times P_{app,oct}^{0{\cdot}631} \quad (r = 0{\cdot}744) \qquad (18)$$

Fig. 4 shows the relationship between the observed values of D_f and the values calculated by use of equation 18. The figure also shows the relationship between the observed values of D_f and the calculated values of D_{fu} estimated from equation 16 multiplied by f_u. Taking f_u into consideration, the correlation coefficient increased from 0·738 to 0·927.

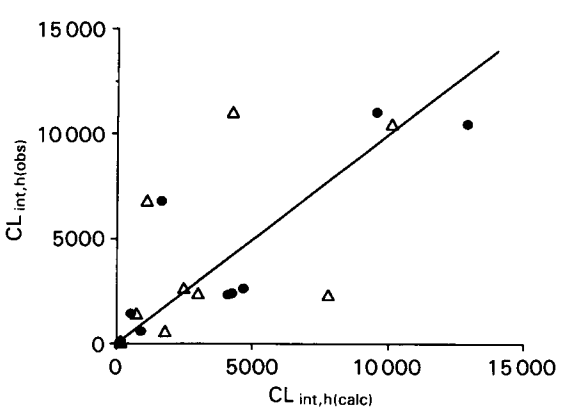

FIG. 3. Relationship between the observed and calculated values of the hepatic intrinsic clearance for ten basic drugs. △ Values calculated using the regression equation for $CL_{int,h,f}$ and $P_{app,oct}$; ● values calculated using the regression equation for $CL_{int,h,fu}$ and P_{oct} and multiplying by f_u. The continuous line indicates a positive correlation.

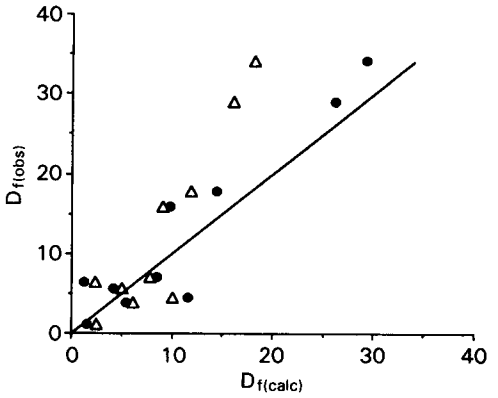

FIG. 4. Relationship between the observed and calculated values of the red blood cell–plasma partition coefficients of ten basic drugs. △ Values calculated using the regression equation for D_f and $P_{app,oct}$; ● values calculated using the regression equation for D_{fu} and P_{oct} and multiplying by f_u. The continuous line indicates a positive correlation.

Discussion

This study has demonstrated that $CL_{int,h}$ and D of highly lipophilic basic drugs can be predicted from P_{oct} by taking f_u into consideration. CL_{urine} determined at steady state in rabbits was considerably smaller than CL_{total}. The percentage contributions of CL_{urine} to CL_{total} were 10% for pentazocine, 3·4% for nitrazepam and less than 2% for other drugs. In the previous study, we reported that the biliary recoveries of intact biperiden (Yokogawa et al 1986) and pentazocine (Ichimura et al 1984) were almost negligible. These findings suggest that the CL_{total} of highly lipophilic basic drugs used in this study is predominantly as a result of hepatic metabolism. Therefore, we investigated the relationship between $CL_{int,h}$ and P_{oct} with regard to the elimination pattern of these drugs.

Hiura et al (1984) has previously reported that for barbiturate drugs there is good correlation between $CL_{int,h,f}$ in the rabbit and the chloroform-water partition coefficient of non-ionized drugs. But as shown in Fig. 1, it was found that log $CL_{int,h,fu}$ of the highly lipophilic basic drugs correlated more highly with log P_{oct} than did log $CL_{int,h,f}$. It is widely known that most basic drugs are catalysed by the metabolic enzyme P-450 in hepatic microsomes. Therefore, the $CL_{int,h}$ of these drugs depends on the fraction of the non-ionized form and relate to the uptake into hepatic cells and the solubility in the lipid bilayer of the smooth endoplasmic reticulum, where these drugs are metabolized (Alberts et al 1994). The pH values in the extracellular and intracellular spaces were 7·4 and 7·0, respectively (Terasaki et al 1984). Moreover, the correlation coefficient of $CL_{int,h,fi}$ estimated from the fraction in the non-ionized form in the intracellular spaces (f_i) by the same method was 0·969, and there was no significant difference when f_u or f_i was used. Therefore, these results could not be used to elucidate factors affecting the metabolic capacity of basic drugs because of the uptake into hepatic cells or the solubility to the lipid bilayer of the smooth endoplasmic reticulum.

The relationship between RBP and drug lipophilicity was investigated by using the D shown in equations 13 and 14. As shown in Fig. 2, the correlation coefficient between D_f and P_{oct} was 0·612 when it was assumed that the unbound drug was taken up by the red blood cells only. Terasaki et al (1984) demonstrated that only the non-ionized form of doxorubicin can penetrate the red-blood-cell membrane. According to the assumption that only the unbound and non-ionized forms of the ten basic drugs are taken up by the red blood cells, there was good correlation between the D_{fu} and P_{oct} ($r = 0·944$). Therefore, for uptake by the red blood cells, for highly lipophilic basic drugs it is necessary to take f_u into consideration.

When the relationships between log $CL_{int,h,fu}$ or log D_{fu} and log P_{oct} are estimated, both the parameters are divided by f_u. Therefore, it might be possible to predict $CL_{int,h,f}$ or D_f even when f_u is not taken into consideration. However, as shown in Figs 3 and 4, the values of $CL_{int,h,f}$ and D_f predicted were closer to the observed values when the regression equation used was that between log $CL_{int,h,fu}$ or log D_{fu} and log P_{oct} rather than that between log $CL_{int,h,f}$ or log D_f and log $P_{app,oct}$.

If the lipophilicity of one basic drug is known, it is possible to predict $CL_{int,h}$, D and K_p (Yokogawa et al 1990). By applying these parameters to a physiologically based pharmacokinetic model, it might be possible to predict the pharmacokinetics of unknown basic drugs.

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