J. Pharm. Pharmacol. 1987, 39: 79–83
Received June 23, 1986



# Plasma protein binding–lipophilicity relationships: interspecies comparison of some organic acids

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Relationships between plasma protein binding of 11 organic acids (benzoic and phenylacetic acid derivatives) and their lipophilicity were studied in man, rabbits, rats and mice. For description, the relationship $f_u = 1/(1 + aD^b)$ was developed, where $f_u$ is the fraction of the unbound drug in plasma, D is the partition coefficient octanol/water and a and b are parameters. While the value of the parameter a is widely different in interspecies comparison, the value of the parameter b is very close in all species studied and is approximately equal to 1. The model used allows the simple calculation of the extent of plasma binding of structurally similar drugs from their lipophilicity, or conversion of the extent of plasma binding from one species to another.

The reversible interaction of drugs and various proteins, particularly plasma albumin, has long been of interest to pharmacologists. In general, only unbound drug is pharmacologically active and capable of diffusing across biological membranes. The extent to which a drug is bound to plasma proteins thus may markedly influence its distribution, rates of metabolism and excretion, and interactions with other drugs (Gillette 1973; Jusko & Gretch 1976; Vallner 1977). Studies on binding to isolated plasma proteins, specifically to albumin, may provide information as to the quantitative binding characteristics, i.e. the number and type of binding sites and association binding constants, and several attempts were made to correlate these binding data quantitatively with the lipophilicity of drugs (Agren et al 1971; Seydel & Schaper 1982). Nevertheless, from the clinical viewpoint, the unbound fraction in whole plasma is related to the pharmacological effect.

The purpose of the present investigation was to study quantitatively the relationships between plasma protein binding and lipophilicity of some model acid drugs using a general valid model enabling simple conversion of the extent of plasma protein binding from the point of view of interdrug and interspecies comparison.

## MATERIALS AND METHODS

### *Drugs*

All iodinated drugs labelled with $^{125}I$ were obtained from the Nuclear Research Centre (Řež, Czechoslovakia) and repurified immediately before use. [carboxy-$^{14}C$]Benzoic acid was obtained from the Institute of Atomic Energy–Isotope Production and Reactor Centre (Otwock-Swierk, Poland). The radiochemical purity was over 97%. [carboxy-$^{14}C$]Salicylic acid (2-hydroxybenzoic acid) was obtained from V/O Izotop (Moscow, USSR). The radiochemical purity was over 97%. [carboxy-$^{14}C$]4-Aminobenzoic acid was obtained from the Isocommerz GmbH (Berlin-Buch, GDR). The radiochemical purity was over 97%. [carboxy-$^{14}C$]4-Acetamino-2-hydroxybenzoic acid was synthesized by the Institute of Macromolecular Chemistry (Prague, Czechoslovakia). The radiochemical purity was over 98%. [carboxy-$^{14}C$]-Tolfenamic acid (*N*-[2-methyl-3-chlorophenyl]-antharanilic acid) was synthesized by the Research Laboratories of the Medica Pharmaceutical Co. Ltd (Helsinki, Finland). The radiochemical purity was over 99%.

### *Plasma protein binding*

Plasma protein binding in-vitro was studied by equilibrium dialysis at 37 °C using the procedure described previously (Lázníček & Květina 1984). Blood samples from healthy subjects or animals (grey Chinchilla strain male rabbits, Wistar strain male rats and Konárovice H-strain male mice) were collected in glass tubes containing dried heparin. The plasma was separated by centrifugation. The plasma (0·5 mL) was dialysed against an equal volume of phosphate-NaCl buffer, pH 7·35 (Ehrnebo et al 1974) with a drug concentration of 1 mg $L^{-1}$. The

* Correspondence.

equilibrium dialysis was carried out for 4 h for all drugs except tolfenamic acid, where the dialysis was carried out for 16 h. The concentrations of drugs were detemined radiometrically with the beta–gamma spectrometer NE 8312 (Nuclear Enterprises Ltd, Edinburgh, UK). Drugs labelled with $^{14}C$ were measured by liquid scintillation counting in Bray's scintillation cocktail (Spolana, Neratovice, Czechoslovakia).

*Partition coefficient octanol/water*

The partition coefficient D for the partitioning of corresponding acids between 1-octanol and water was determined as follows: The drug (1–10 mg $L^{-1}$) in 0·5 M HCl was shaken with an equal volume of 1-octanol. After centrifugation, a part of the organic phase with the drug was agitated and equilibrated with an equal volume of 0·5 M HCl. The drug concentrations in organic and aqueous phases were determined radiometrically. For 4-aminobenzoic acid, where protonization of an amino-group in acid solutions occurs, the partition coefficient was determined as $D = 2D_C$, where $D_C$ is the distribution ratio octanol/water at pH = $pK_a$ of the acid carboxyl group.

*Plasma binding–lipophilicity relationships*

It is generally accepted that albumin is the major protein in the body responsible for the non-specific binding of weakly acidic drugs. The plasma protein binding data, assuming binding only to albumin, will obey an equation of the Scatchard form (Scatchard 1949):

$$\frac{C_B}{C_P} = \sum_{i=1}^{m} \frac{n_i k_i C_F}{1 + k_i C_F}, \qquad (1)$$

where $C_B$, $C_F$ and $C_P$ are molar concentrations of the bound drug, free drug and proteins, respectively, $n_i$ is the number of sites in class i, and $k_i$ is the site binding constant for the i-th class (Hunston 1975).

If the concentration of a drug is very low and $k_i C_F$ is $\ll 1$, equation (1) can be simplified to

$$\frac{C_B}{C_P C_F} = \sum_{i=1}^{m} n_i k_i = K, \qquad (2)$$

where K is the equilibrium binding constant. (The question is up to what concentration of a drug it is acceptable to neglect the term $k_i C_F$. For example, if the total concentration of a drug is $5 \times 10^{-6}$ M, it is possible to estimate according to Solomon & Thomas (1971) that for any $k_i$ there is the value of $k_i C_F < 0{\cdot}01$.)

Equation (2) can be rearranged

$$\frac{1 - f_u}{f_u C_P} = K, \qquad (3)$$

where $f_u$ is a fraction of the free drug in plasma, $f_u = C_F/(C_B + C_F)$.

The relationship between the equilibrium binding constant and lipophilicity (characterized by the partition coefficient octanol/water, D) follows from chemical thermodynamics in the form (Chien et al 1975)

$$\log K = \frac{\mu^0_{org} - \mu^0_{prot}}{2{\cdot}303\,RT} + \log D, \qquad (4)$$

where $\mu^0_{org}$ and $\mu^0_{prot}$ are the standard chemical potentials for a drug species in octanol and bound to protein molecule, respectively.

Substituting equation (3) for the K term in equation (4) and rearranging to give:

$$\log\left(\frac{1}{f_u} - 1\right) = \frac{\mu^0_{org} - \mu^0_{prot}}{2{\cdot}303\,RT} - \log C_P + \log D \qquad (5)$$

may be simplified to

$$\log\left(\frac{1}{f_u} - 1\right) = c + b \log D \qquad (6)$$

and rearranged to

$$f_u = \frac{1}{1 + aD^b}, \qquad (7)$$

where a, b and c are constants.

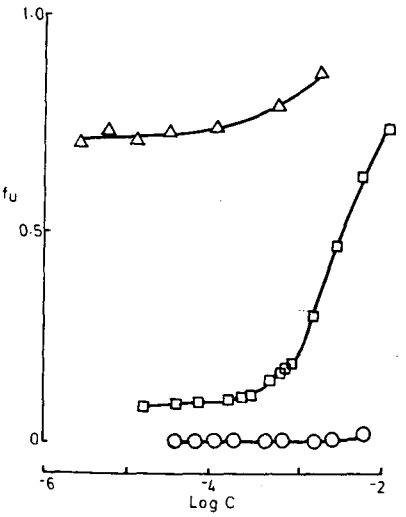


FIG. 1. Free drug fraction in human plasma as a function of its total plasma concentration: ○, tolfenamate; □, salicylate; and △, 4-aminobenzoate.

Table 1. Free drug fraction in plasma and partition coefficient octanol/water of model drugs under study. (Means ± s.d.)

| Compound | Free drug fraction × 100 ($f_u \times 100$) | | | | log D |
|---|---|---|---|---|---|
| | Human plasma | Rabbit plasma | Rat plasma | Mouse plasma | |
| Benzoate | 16·3 + 1·3 | 26·6 + 1·1 | 55·9 + 7·0 | 75·4 + 6·5 | 1·94 |
| 2-Iodobenzoate | 8·2 + 1·0 | 14·5 + 1·7 | 42·1 + 5·3 | 65·4 ± 4·6 | 2·32 |
| 3-Iodobenzoate | 3·8 + 0·7 | 5·6 + 1·2 | 6·6 + 1·4 | 10·3 + 0·5 | 2·68 |
| 4-Iodobenzoate | 3·1 + 0·9 | 3·7 + 0·6 | 6·1 + 1·9 | 9·7 + 1·3 | 2·81 |
| Salicylate | 6·3 + 0·2 | 8·2 + 0·9 | 21·8 + 6·4 | 41·8 + 3·7 | 2·24 |
| 4-Aminobenzoate | 70·4 + 5·2 | 76·6 + 13·9 | 87·5 + 1·3 | 91·0 + 5·8 | 0·74 |
| 4-Acetamino-2-hydroxy-benzoate | 19·6 + 2·2 | 20·8 + 2·1 | 48·2 + 2·0 | 65·4 + 3·0 | 1·62 |
| Tolfenamate | 0·08 + 0·01 | 0·089 + 0·016 | 0·30 + 0·02 | 0·45 + 0·03 | 4·18 |
| 2-Iodophenylacetate | 5·6 + 0·4 | 6·0 + 1·4 | 27·5 + 5·0 | 66·0 + 7·5 | 2·25 |
| 3-Iodophenylacetate | 2·3 + 0·5 | 2·7 + 0·2 | 7·4 + 0·6 | 30·4 + 1·4 | 2·49 |
| 4-Iodophenylacetate | 1·9 + 0·6 | 2·7 + 0·1 | 12·3 + 1·0 | 15·1 + 2·8 | 2·67 |

## RESULTS

Typical examples of free fraction-drug concentration dependence are shown in Fig. 1. At low drug levels (up to the concentration of total drug about $10^{-4}$ M, when less than 20% of albumin molecules are occupied by a drug) the change in binding is modest, but then an increase in the unbound fraction occurs.

Table 1 lists the results of plasma protein binding experiments together with the lipophilicity characterized by the partition coefficient octanol/water. Considerable interspecies differences exist in plasma binding of model drugs studied. For statistical evaluation, plasma binding–lipophilicity correlations were fitted to equation 6 by linear least squares regression analysis. Quantitative relationships are given in the equations:

For man: $\log (1/f_u - 1) = -1{\cdot}096 + 0{\cdot}994 \log D$ (8)
$n = 11, r = 0{\cdot}985, s = 0{\cdot}155, F = 293{\cdot}5$

For rabbits: $\log (1/f_u - 1) = -1{\cdot}275 + 1{\cdot}016 \log D$ (9)
$n = 11, r = 0{\cdot}972, s = 0{\cdot}218, F = 153{\cdot}5$

For rats: $\log (1/f_u - 1) = -1{\cdot}745 + 1{\cdot}011 \log D$ (10)
$n = 11, r = 0{\cdot}965, s = 0{\cdot}243, F = 122{\cdot}9$

For mice: $\log (1/f_u - 1) = -2{\cdot}151 + 1{\cdot}035 \log D$ (11)
$n = 11, r = 0{\cdot}940, s = 0{\cdot}332, F = 68{\cdot}9$

In equations 8–11, n is the number of data points used in analysis, r is the correlation coefficient, s is the standard error of estimate and F is the F-test;

Parameters of the relationships between the free fraction and lipophilicity described by equation 7 are listed in Table 2.

For a graphical illustration a more suitable plot of unbound fraction in plasma versus the logarithm of lipophilicity is demonstrated in Fig. 2. Theoretical plots from individual species form sigmoidal curves with an approximately coincidental slope, but they are mutually shifted relative to the value of log D.

## DISCUSSION

Interspecies comparison of plasma protein binding of drugs under study could be expressed in terms of different values of the characteristics a and b in equation (7). This equation adequately describes protein binding over the whole range of possible drug lipophilicity and predicts the maximum and minimum free fraction a drug can achieve. This property may appear to be trivial, but it is not shared by the linear or quadratic model correlating the unbound fraction (or logarithm of the unbound fraction) versus the logarithm of lipophilicity (Lucek & Coutinho 1976; Lázníček et al 1985). The applicability of equation 7 for the evaluation of protein binding data presented in this paper is qualified by the presumption that binding to plasma proteins other than albumin is negligible. The major plasma

Table 2. Interspecies comparison of predicted parameters of plasma binding–lipophilicity relationships.

| Species | $f_u = \frac{1}{1 + aD^b}$ | |
|---|---|---|
| | a | b |
| Human plasma | 0·08016 | 0·994 |
| Rabbit plasma | 0·05313 | 1·016 |
| Rat plasma | 0·01793 | 1·011 |
| Mouse plasma | 0·00706 | 1·035 |

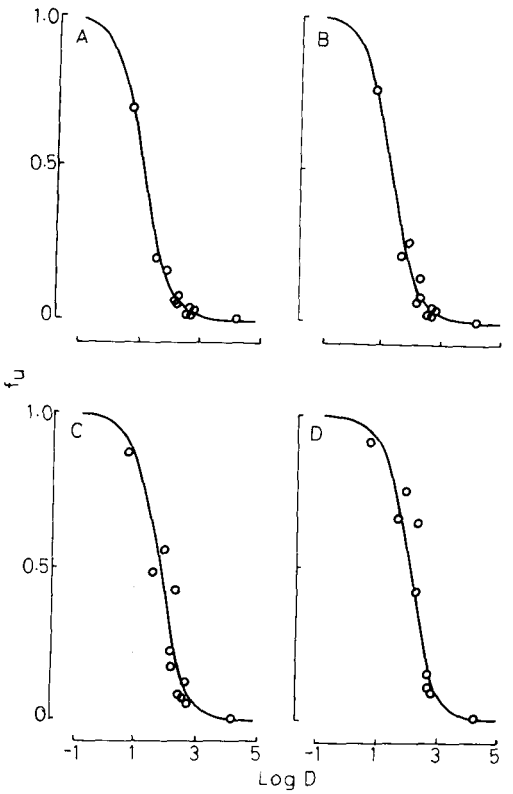


FIG. 2. Free drug fraction/lipophilicity plots for human plasma (A), rabbit plasma (B), rat plasma (C) and mouse plasma (D).

proteins involved in drug binding are albumin and $\alpha_1$-acid glycoprotein. Anionic drugs are assumed to be bound to albumin (Settle et al 1971), while $\alpha_1$-acid glycoprotein is the main reaction partner of basic drugs in drug-protein interactions (Piafsky 1980). Even when a limited number of acid drugs are bound to $\alpha_1$-acid glycoprotein, the drugs exhibiting carboxylic groups are either not, or only poorly bound to that glycoprotein (Urien et al 1982). For this reason the presumption of exclusive binding of the drug studied only to albumin seems to be valid.

Our results demonstrate substantial interspecies differences in plasma binding of studied organic acids. The order of binding was human plasma > rabbit plasma > rat plasma > mouse plasma and the correlation coefficient of equations 8–11 decreased in the same order. The degree of drug binding to plasma proteins is given as a result of hydrophobic bonding due initially to electrostatic attraction reinforced by hydrogen bonds and Van der Waals' forces. On the other hand, the partition coefficient does justice only to the hydrophobic properties of the drug molecule. When binding to albumin of different species is compared, a decrease in the hydrophobic binding forces probably contributes to an accentuation of other forces involved in binding of drugs to albumin, which leads to a weaker correlation between binding and lipophilic characteristics.

In Table 2, the value of the parameter a is widely different in interspecies comparison, while the value of the parameter b is very close in all species studied and approaches the theoretical value, given by equation (4).

The free fraction of a drug in plasma has a profound effect on its pharmacokinetics both for distribution (Wilkinson & Shand 1975) and rate of elimination (Gibaldi et al 1978); our results make possible a simple calculation of the free drug fraction in plasma of structurally similar drugs from their known partition coefficients (which are for most drugs given in the literature) as follows:

$$f_u = (1 + a\,D)^{-1}, \qquad (12)$$

because the value of the parameter b is not significantly different from unity and is eliminated from the expression.

For conversion of the results of protein binding experiments among species, the calculation may be made by equation

$$\frac{1}{f_{uA}} = 1 + \frac{a_A}{a_B}\left(\frac{1}{f_{uB}} - 1\right) \qquad (13)$$

where A and B indicate the values for species A and B, respectively. On the other hand, equation 13 also allows the calculation of the ratio $a_A/a_B$ from the known free fraction of one drug in the plasma of two different species.

*Acknowledgements*

The authors wish to thank the firm Medica Pharmaceutical Co. Ltd and the Institute of Macromolecular Chemistry, Prague for the kind donation of radiolabelled drugs. The authors also wish to thank Dr B. Mánek for language correction.

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