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The effect of lipophilicity on the protein binding and blood cell uptake of some acidic drugs

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Abstract

Quantitative relationships between lipophilicity (characterized by the octanol–water partition coefficient) and binding to both human plasma proteins and blood cells have been studied in a group of model anionic drugs (benzoic and phenylacetic acid derivatives). Protein binding in plasma and accumulation in blood cells in suspension increases with increasing lipophilicity. For quantitative evaluation, the equation $\log R = a + b \log D$ has been employed, where R is the bound-to-free drug ratio, D is lipophilicity, and a and b are constants. Whereas the protein bound-to-free drug ratio is proportional to drug lipophilicity, the cell bound-to-free drug ratio correlates with lipophilicity to the power 0.685. Distribution in whole blood is affected by protein binding and also by cell accumulation. In blood, the free drug fraction and the fraction in blood cells decrease with increasing lipophilicity, whereas the protein-bound fraction correspondingly increases.

Keywords: Benzoates; Blood cell uptake; Blood distribution; Lipophilicity; Phenylacetates; Protein binding

1. Introduction

After entering the bloodstream, drugs are to some extent bound to plasma proteins, accumulated in blood cells and, in part, are dissolved in plasma water. Only this latter, unbound part is thought to diffuse across biological membranes. That is why the proportion of unbound drug influences drug distribution, rate of elimination and concentration at the receptor site, as discussed by Gillette [1] and others [2–4].

In a previous work, a theoretical relationship was derived between lipophilicity and the proportion of unbound drug in a group of model acidic drugs [5]. In this report, the effect of lipophilicity on protein binding, blood cell accumulation and blood partitioning in some benzoic and phenylacetic acid derivatives is examined. The present work tests the possibility of evaluating the blood distribution of studied compounds based on their physicochemical characteristics.

2. Experimental

2.1. Labelled compounds

(^{14}C) benzoic acid and (^{14}C) phenylacetic acid (Inst. Atomic Energy-Isotope Production, Otwock-Swierk, Poland) had radiochemical purities of over 97%; (^{14}C) salicylic acid (V/O Izotop, Moscow, USSR)) had a radiochemical purity of over 97%; (^{14}C) tolfenamic acid (*N*-(2-Methyl-3-chlorophenyl)-anthranilic acid) was synthesized by the Research Laboratories of Medica Pharmaceutical Co. Ltd (Helsinki, Finland), and had a radiochemical purity over

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99%. All these acids were used in the form of the corresponding salts. Compounds labelled with ^{125}I (2-iodobenzoate, 3- iodobenzoate, 4-iodobenzoate, 2-iodophenylacetate, 3-iodophenylacetate, and 4-iodophenylacetate) were obtained from the Nuclear Research Centre (Řež, Czech Republic) and were repurified immediately before use.

2.2. Determination of plasma protein binding

Binding of the compounds under study to human plasma obtained from healthy subjects was studied in vitro by equilibrium dialysis at 37 °C using the procedure described previously [6]. The initial drug concentration was 1 mg ml^{-1}.

2.3. Determination of blood cell accumulation

The cell fraction from fresh heparinized human blood was washed four times with isotonic phosphate–NaCl buffer (pH 7.35) [7] and the final volume of the suspension was adjusted to the initial volume of the blood sample (hematocrit 0.40). The blood cell suspension was then incubated with the compounds (concentration 1 mg l^{-1}) at 37 °C until equilibrium was reached (2 h). The distribution ratio between blood cells and the supernatant was then determined.

2.4. Blood partitioning

Fresh heparinized whole human blood was incubated with model compounds (concentration 1 mg l^{-1}) at 37 °C until equilibrium (2 h). The drug concentration in blood cells and plasma was measured and the plasma protein binding of each drug was determined.

2.5. Lipophilicity determination

Lipophilicity was determined as the logarithm of the partition coefficient D of each acid between 1-octanol and 0.5 M HCl, as described previously [5].

2.6. Radioactivity determination

The radioactivity of the samples of whole blood, plasma, and blood cell suspension was determined either by liquid scintillation counting with an 1219 Rackbeta (LKB Wallac) using external standard quench fitting or by gamma counting with a gamma-spectrometer Tesla (Tesla, Liberec).

2.7. Calculations

The drug fraction in whole blood distributed to plasma water (λ_{PW}), to the plasma proteins (λ_{PP}), and to the blood cells (λ_{BC}), and the drug fraction in the blood cell suspension distributed to the blood cells (λ_{BCs}) were calculated as follows:

$$\lambda_{PW} = C_P f_u (1 - H)/C_B \quad (1)$$

$$\lambda_{PP} = C_P (1 - f_u)(1 - H)/C_B \quad (2)$$

$$\lambda_{BC} = 1 - \lambda_{PW} - \lambda_{PP} \quad (3)$$

$$\lambda_{BCs} = 1 - [C_{BUs}(1 - H)/C_{BCs}] \quad (4)$$

where C_P is the total drug concentration in plasma, C_B is the total drug concentration in whole blood, H is the haematocrit, f_u is the free drug fraction in plasma, C_{BUs} is the drug concentration in the buffer phase of the blood suspension, and C_{BCs} is the total drug concentration in the blood cell suspension.

The value of C_B was determined either by direct measurement or, in the case of a very low λ_{BC} value, by use of the equation

$$C_B = C_P(1 - H) + C_{BCm}H \quad (5)$$

where C_{BCm} is the drug concentration in the blood cell mass corrected for the remainder of the plasma (or buffer) in the cell mass.

The drug binding ratio (protein bound-to-free drug ratio) in plasma (R_P) and the drug partition ratio between blood cells and buffer phase of blood cell suspension (P_{BC}) were calculated as follows:

$$R_P = (1 - f_u)/f_u \quad (6)$$

$$P_{BC} = C_{BCm}/C_{BUs} \quad (7)$$

If it is assumed that at equilibrium the free drug concentration in plasma (buffer) is equal to that in the water phase of the blood cell mass, the blood cell binding ratio (blood cell bound-to-free drug ratio) (R_{BC}) can be calculated as

$$R_{BC} = P_{BC} - P_{water} \quad (8)$$

where P_{water} is the water partition ratio between blood cells and the buffer phase. P_{water} was determined with tritium radiolabeled water, giving a value of 0.72.

Table 1
Free drug fraction in plasma (f_u), blood cell binding ratio (R_{BC}) and lipophilicity (log D) of studied compounds

Compound	f_u	R_{BC}	log D
Benzoate	0.163 ± 0.013	0.58 ± 0.22	1.94
2-Iodobenzoate	0.082 ± 0.010	0.88 ± 0.25	2.32
3-Iodobenzoate	0.038 ± 0.007	1.79 ± 0.21	2.68
4-Iodobenzoate	0.031 ± 0.009	1.79 ± 0.11	2.81
Salicylate	0.063 ± 0.007	0.84 ± 0.25	2.24
Tolfenamate	0.0008 ± 0.0001	19.2 ± 3.2	4.18
Phenylacetate	0.192 ± 0.018	0.52 ± 0.19	1.92
2-Iodophenylacetate	0.056 ± 0.004	0.93 ± 0.28	2.25
3-Iodophenylacetate	0.023 ± 0.005	1.58 ± 0.20	2.49
4-Iodophenylacetate	0.019 ± 0.006	0.93 ± 0.32	2.67

The relationship between the drug binding ratio in plasma and lipophilicity follows from chemical thermodynamics in the form [8]

$$\log R_P = a + b \log D \quad (9)$$

where a and b are constants.

When a similar approach is applied to the drug binding to blood cells, the relationship between the blood cell binding ratio and lipophilicity will obey the following relationship:

$$\log R_{BC} = c + d \log D \quad (10)$$

where c and d are constants.

If the values of a, b, c and d are estimated, the theoretical drug distribution in blood at equilibrium as a function of lipophilicity can be calculated according to the equations

$$\lambda_{PW} = F(1 - H) \quad (11)$$

$$\lambda_{PP} = F(1 - H) \cdot 10^{(a + b \log D)} \quad (12)$$

where

$$1/F = 1 - H(1 - P_{water}) + (1 - H) \cdot 10^{(a + b \log D)} + H.10^{(c + d \log D)} \quad (13)$$

3. Results

Distribution characteristics of the studied organic acids in human blood are summarized in Table 1. For the evaluation of relationships between lipophilicity and drug binding ratio in plasma or in blood cells, Eqs. (9) and (10) were employed. Quantitative relationships are given in the equations

$$\log R_P = -1.246 + 1.042 \log D \quad (14)$$
$(n = 10,\ r = 0.947,\ s = 0.168,\ F = 145.6)$

$$\log R_{BC} = -1.588 + 0.685 \log D \quad (15)$$
$(n = 10,\ r = 0.994,\ s = 0.050,\ F = 698.4)$

where n is the number of data points used in the statistical analysis, r is the correlation coefficient, s is the standard error of the estimate and F is the F-test value.

For a graphical illustration, a more suitable plot of unbound fraction in plasma against lipophilicity is demonstrated in Fig. 1; the distribution between blood cells and buffer phase against lipophilicity (calculated by the combination of Eqs. (8) and (15) is shown in Fig. 2.

Experimental and theoretical values of distributions of compounds under study in whole blood are listed in Table 2; the distribution-lipophilicity profile is illustrated in Fig. 3.

4. Discussion

The clinical significance of drug binding to various blood proteins is well documented. Drugs are bound in plasma to different proteins, but two proteins are responsible for most binding reactions, namely albumin and α_1-acid glycoprotein (orosomucoid). Albumin (MWt 69 000 D) is thought to bind acidic drugs whereas α_1-acid glycoprotein (MWt 44 000 D) binds predominantly basic drugs. Albumin is highly concentrated (about 580 μM) whereas α_1-acid glyco- protein is present in plasma in much lower concentration (about 23 μM). Even if some acidic drugs may also interact with α_1-acid glycoprotein, drugs with carboxylic groups are poorly bound or not bound to this protein [9]. Human albumin is known to have two major drug binding sites [10] which are stereoselective [11]. For the drug concentrations used in the experiments, at equilibrium, less

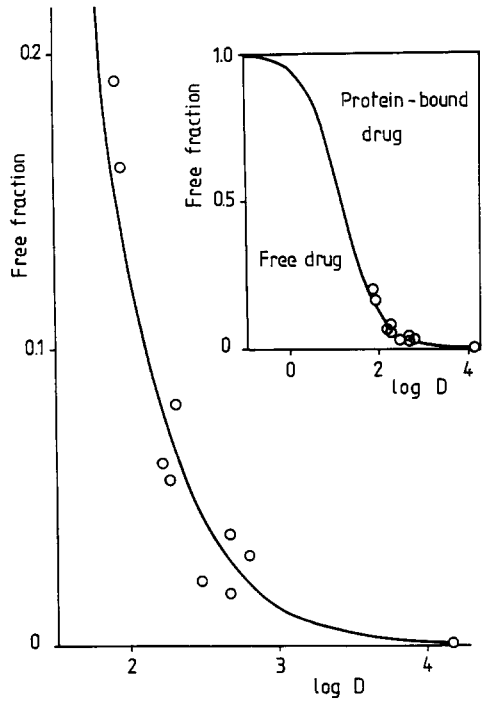

Fig. 1. Plot of free drug fraction in human plasma against lipophilicity of organic acids under study.

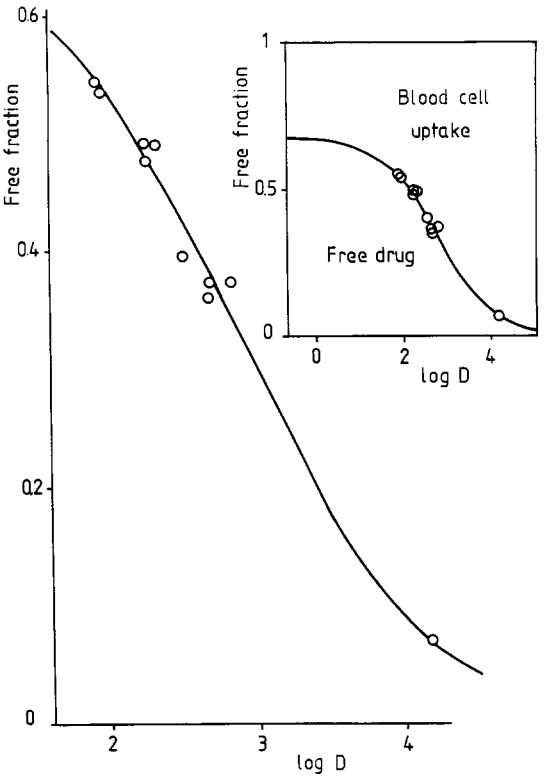

Fig. 2. Effect of lipophilicity on blood cell uptake of studied acidic drugs in human blood cell suspension, haematocrit 0.40.

than 1% of albumin molecules were occupied; from such a small drug-to-albumin ratio, no significant influence of the extent of protein binding on drug concentration can be expected. Drugs are generally bound to albumin in several different ways, namely by ionic bonding, hydrophobic bonding, hydrogen bonding and van der Waals forces [12]; the extent of binding results from a combination of the effects of all these forces.

The octanol–water partition coefficient is a measure of the lipophilicity of compounds and thus can serve as an effective physicochemical parameter for estimates of hydrophobic interactions between a drug molecule and protein. For this reason, significant relationships between lipophilicity and the extent of protein binding can be achieved only in a group of compounds for which the contribution of forces other than hydrophobic bonds in a drug–protein interaction is similar. This is the

Table 2
Experimental and calculated values of distributions of the compounds under study in whole blood (haematocrit 0.40)

Compound	Fraction in plasma water, λ_{PW}		Fraction bound to plasma proteins, λ_{PP}		Fraction distributed to blood cells, λ_{BC}	
	Exp	Calc	Exp	Calc	Exp	Calc
Benzoate	0.14	0.128	0.73	0.763	0.13	0.109
2-Iodobenzoate	0.07	0.059	0.83	0.874	0.10	0.067
3-Iodobenzoate	0.04	0.026	0.89	0.929	0.07	0.045
4-Iodobenzoate	0.03	0.020	0.91	0.942	0.06	0.038
Salicylate	0.06	0.070	0.88	0.854	0.06	0.076
Tolfenamate	< 0.01	0.001	0.98	0.989	0.02	0.010
Phenylacetate	0.16	0.133	0.69	0.756	0.15	0.111
2-Iodophenylacetate	0.05	0.068	0.88	0.858	0.07	0.074
3-Iodophenylacetate	0.02	0.041	0.94	0.906	0.04	0.053
4-Iodophenylacetate	0.02	0.027	0.95	0.928	0.03	0.045

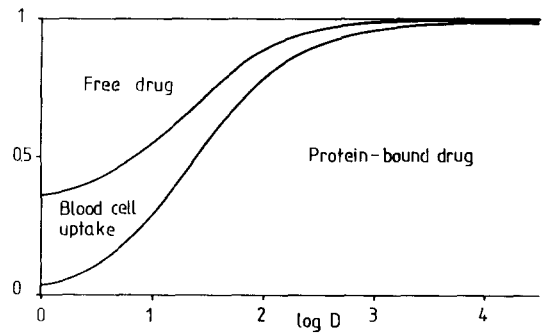

Fig. 3. Plot of theoretical distribution against lipophilicity of studied organic acids in human blood, haematocrit 0.40.

case for the compounds under study in this paper where all the compounds are weak acids (derivatives of benzoic and phelylacetic acids), which are practically completely ionized at physiological values of pH (analogous ionic bonds and van der Waals forces in interaction with protein); in addition, for most of these acids other structural characteristics are similar. The relationship between the drug binding ratio in plasma and lipophilicity given by Eq. (14) confirms the above presumption.

The accumulation and transport of organic compounds in biological structures depends largely on the distribution of the molecules between the lipid and aqueous phases, which in turn correlates with the octanol–water partition coefficient. To explain the accumulation of drugs in blood cells, it is postulated that transport of carboxylic acid derivatives across the human blood cell membrane is due to passive diffusion of non-ionic species across the lipid interior of the membrane [13]. As expected, the binding ratio of the compounds under study to blood cells depends on the lipophilicity and this relationship is described by Eq. 15. Unlike the binding to proteins, where the partition coefficient of the acid seems to be the most adequate parameter for the estimation of hydrophobic interactions between the drug molecule and protein, the distribution coefficient determined at pH 7.4 is probably a more suitable parameter for the description of the accumulation of drugs in the lipid phase of blood cells. Drugs can enter blood cells, or adhere to the surfaces of blood cells, or do both [14]; in addition, binding to blood cell proteins could also result in cell binding. As the distribution coefficient closely correlates with the partition coefficient in the group of model drugs under study, the relationship between blood cell accumulation and the partition coefficient was used in the present work because this approach makes it possible to calculate the relationships between physicochemical and binding characteristics in whole blood.

In both plasma and blood cell suspension the free fraction decreases with increasing lipophilicity. In the binding to plasma proteins the binding ratio approximately correlates with the octanol–water partition coefficient; in contrast the binding ratio to blood cells was correlated with the octanol–water partition coefficient to the power 0.685. This is the reason for the increase in the relative proportion of the compounds bound to plasma proteins and for the decrease both in blood cell uptake and proportion of unbound drug in whole blood with increasing lipophilicity (Fig. 3). At low lipophilicity values the drug is mostly in the free form and distributed to blood cells whereas at high lipophilicity values most of the compound is bound to plasma proteins. The equations derived adequately describe blood distribution over a whole range of possible drug lipophilicity, but of course these equations are not valid generally for all types of drugs. The requirement of structure similarity is necessary for the valid determination of drug distribution characteristics in blood based on Eq. (13) and (14).

In conclusion, it has been demonstrated in a group of anionic drugs (benzoic and phenylacetic acid derivatives) that both plasma protein binding and blood cell uptake depend on lipophilicity (determined as the octanol–water partition coefficient of the acid). This physicochemical parameter can be used for the prediction of the blood distribution of structurally similar drugs.

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