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Research Article

Relationships in the Structure–Tissue Distribution of Basic Drugs in the Rabbit

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The relationship between the tissue-to-plasma partition coefficients (K_p) and drug lipophilicity was investigated using highly lipophilic drugs with apparent partition coefficients of 150 or above in an octanol–water system at pH 7.4. Ten clinically popular basic drugs with different dissociation coefficients (pK_a) and lipophilicity were used. The K_p values were determined in nondisposing organs after the i.v. administration of individual drugs in rabbits. The free fraction in plasma and the blood-to-plasma concentration ratio were determined *in vitro*. Then the tissue-to-plasma ratios of nonionized and unbound drug concentrations (K_{pfu}) were calculated from K_{pf} (ratio of unbound drug). The true octanol–water partition coefficient of the nonionized drugs (P) was used to analyze the K_{pf} and K_{pfu}. In all tissues, log K_{pfu} was more highly correlated with log P than log K_{pf}.

KEY WORDS: basic drugs; tissue distribution; lipophilicity; rabbits; distribution of nonionized drug; pharmacokinetics.

INTRODUCTION

Basic lipophilic drugs are distributed in large apparent volumes, and their relative lipophilicity may be an important property in distinguishing these drugs (1). Lipophilicity affects their pharmacokinetic parameters such as clearance (2) and volume of distribution (1,3–7). At present, two approaches are used to analyze the relationship between distribution volume and lipophilicity. First, the apparent organic solvent–water partition coefficients at pH 7.4 (APC) are used as an index to the relationship between APC and the volumes of distribution for acidic (1) and basic drugs (1,3–5). Second, the partition coefficients (P) of nonionized form are used to analyze the distribution volumes of acidic drugs (6,7).

In general, serum protein binding affects tissue drug distribution. The distribution volumes of basic drugs have been determined in relation to their plasma protein binding in humans (8–10). In earlier studies (11–13), we constructed a physiological pharmacokinetic model for the basic drug biperiden using the tissue-to-plasma concentration ratio (K_{pf}) of unbound drugs. However, little information is available concerning the comparative distribution behavior of the basic drugs and the mechanism of tissue distribution in relation to both their lipophilicity and their protein binding parameters. The exact relationship between the lipophilicity and the tissue-to-plasma partition coefficient (K_p) has not been established yet.

According to the pH partition hypothesis, only nonionized, unbound drugs penetrate the plasma membrane, and at equilibrium, the concentration of the nonionized species is the same on both sides of the membrane (14). Most of the evidence supporting the pH partition hypothesis stems from studies of gastrointestinal absorption, renal excretion, and gastric secretion of drugs (15) and indicates that only the nonionized form of the drug is dissolved in the plasma membrane. To quantify the relationship between lipophilicity and tissue distribution of basic drugs, the concentration of nonionized drug in distribution sites must be taken into account. We therefore hypothesized that only the nonionized form free from plasma protein binding can diffuse through the plasma membrane to establish equilibrium between the drug in the tissue lipophilic compartment and the nonionized form of the drug in the intracellular fluid. The ratio of the drug concentration in tissue to the nonionized form in intracellular fluid may be correlated with the lipophilicity.

To examine this hypothesis, we conducted a study in rabbits using highly lipophilic drugs with apparent partition coefficients of 150 or above in an octanol–water system at pH 7.4. Ten basic drugs with different pK_a, lipophilicity, and plasma protein binding were studied. The values of K_p were determined in various tissues at steady state. Then we calculated the ratio (K_{pfu}) of the concentration of nonionized and unbound drugs in tissue to the concentration in plasma. The index of lipophilicity of the drugs used was log P in the octanol–water system. We found the overall values of log K_{pfu} were highly correlated with the log P in each tissue.

MATERIALS AND METHODS

Materials. Biperiden, haloperidol (Dainippon, Osaka,

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Japan), chlorpromazine, clotiazepam (Yoshitomi, Osaka, Japan), clomipramine (Ciba Geigy, Japan), diazepam, trihexyphenidyl (Takeda, Osaka, Japan), nitrazepam, promethazine (Shionogi, Osaka, Japan), and pentazocin (Sankyo, Tokyo) were used as supplied. All other chemicals were of reagent grade and were used without purification.

Animal Experiments. Adult male albino rabbits weighing 2.1 ± 0.2 kg were used randomly in this study. Animal experiments were carried out in essentially the same way as described previously (16,17). Briefly, under light anesthesia the femoral artery and vein were cannulated with polyethylene tubing. The dosage of biperiden used in this study, 3.2 mg/kg, was based on the previous studies (12). A 0.24-ml portion of the drug solution was injected over a period of 2 min through the femoral vein cannula. Blood samples were withdrawn from the femoral artery at designated time intervals and collected in heparinized tubes. The plasma was separated by centrifugation and stored at −30°C until assayed.

To determine the tissue-to-plasma partition coefficient in the steady state, infusion studies were performed to obtain the plasma concentration ranging between 100 and 200 ng/ml. The priming and maintenance doses were calculated based on preliminary determination of the values of the distribution volume and clearance for each drug after an i.v. bolus administration. For example, biperiden was infused at a rate of 0.180 ml/hr (5.32-mg/ml drug solution) after intravenous bolus injection of the priming dose of 3.2 mg/kg, since the volumes of distribution and clearance in rabbits were 19.3 liters/kg and 78.7 ml/min/kg, respectively. At 16 hr after the infusion studies began, the rabbits were sacrificed for tissue sampling. The procedure for preparing tissue homogenate was essentially similar to that described previously (16,17).

Determination of Serum Protein Binding. The extent of drug binding to rat serum protein was measured in each group by the equilibrium dialysis technique with a sample volume of 0.8 ml as described previously (12).

pK_a and Lipophilicity. The pK_a values were determined by the potentiometric titration at 37°C (18). The organic solvent–water partition coefficients of drugs were determined experimentally at 37°C. Benzene, chloroform, octanol, or triolein was used as the organic solvent, and isotonic phosphate buffer (pH 7.4) was used as an aqueous solution. To minimize the volume change due to mutual miscibility, aqueous and organic phases were presaturated with each other. An exactly measured amount (3–100 ml) of each solution was transferred to a siliconized glass-stoppered flask and shaken for 16 hr at 37°C to achieve complete equilibrium. The two phases were separated by allowing the flask to stand for 1 hr, and the aqueous phase was centrifuged at 3000 rpm for 10 min, then extracted into ether and assayed. The apparent partition coefficients (P_{app}) at pH 7.4 were calculated by dividing the concentration in the organic phase by the concentration in the aqueous phase without correction for ionization. The true partition coefficient (P) for nonionized drugs was calculated from the apparent partition coefficient according to the Henderson–Hasselbalch equation.

Assay for Drugs. Drug concentrations in plasma and various tissue homogenates were determined by gas–liquid chromatography (GLC) as described previously (19). Briefly, homogenized tissues and/or plasma were mixed with portions of 1 M Na_2CO_3 buffer (pH 10.0), an internal standard was added, then the samples were extracted with diethyl ether, and centrifuged for 5 min at 2000 rpm. The organic phase was then transferred into another series of test tubes, each containing 2 ml of 1 N HCl. The drug was reextracted into the HCl, and the resulting ether phase was discarded. Then 1 ml of 3 N NaOH was added to the aqueous phase, together with 5 ml of diethyl ether. The final ether phase was transferred to a glass centrifuge tube and evaporated. The dried residue was dissolved in 20 μl of diethyl ether, and most of the solution was applied to the chromatograph.

Data Analysis. The data were analyzed using a digital computer, FACOM-M360AP, at the Information Processing Center, Kanazawa University, with Fisher's t test used to compare the unpaired means of two sets of data. Analysis of variance (ANOVA) was used to compare more than two sets of data. The number of determinations (N) is noted in the tables.

RESULTS

Partition Coefficients and pK_a. Ten clinically popular basic drugs with different pK_a's were selected as listed in Table I. For comparison, the apparent partition coefficients

Table I. Physicochemical Properties of Ten Basic Drugs

				Apparent partition coefficient at pH 7.4, at 37°C				log P (octanol–water partition coefficient of nonionized form)			
Key	Substrate	pK_a	MW	Octanol	Benzene	Chloroform	Triorein	Octanol	Benzene	Chloroform	Triorein
1	Pentazocin	8.5	285.4	150	165	203	129	3.31	3.35	3.44	3.24
2	Nitrazepam	3.4	281.3	162	39	262	51	2.21	1.59	2.42	1.71
3	Haloperidol	7.8	375.9	485	598	6,910	10,400	3.23	3.32	4.39	4.56
4	Biperiden	8.8	311.5	678	6,020	22,100	20,800	4.25	5.20	5.76	5.74
5	Diazepam	3.5	284.8	970	4,900	27,900	3,780	2.99	3.69	4.45	3.58
6	Promethazine	9.1	284.4	1,270	4,010	13,900	30,800	4.81	5.31	5.85	6.20
7	Trihexyphenidyl	8.7	337.9	1,470	7,630	28,300	11,600	4.49	5.20	5.77	5.38
8	Chlorpromazine	9.3	318.9	1,900	2,720	3,630	8,640	5.19	5.34	5.49	5.84
9	Clotiazepam	3.6	318.8	3,060	11,500	29,700	75,100	3.49	4.06	4.47	4.88
10	Clomipramine	8.5	314.9	3,800	14,700	54,600	233,000	4.71	5.30	5.87	6.50

were determined with four kinds of organic solvent. The values of log P varied widely (Table I). Triolein varied most.

Plasma Protein Binding. The unbound fractions of drugs in serum (f_p) are listed in Table II. Binding to rabbit serum within the series varied widely with f_p, which ranged from 0.03 to 0.40.

Tissue-to-Plasma Partition Coefficient (K_p) at Steady State. After a drug was infused i.v. at a constant rate, the K_p's for nondisposing organs were calculated from the steady state concentrations in the tissue and plasma (Table II). K_p is defined as the ratio of the concentration in the tissue to the concentration in the plasma. The reported K_p values for rabbit are also cited in Table II (11,12).

Model Development and the Comparison. The apparent volume of distribution in humans has been predicted for many basic drugs from animal data regarding the modified unbound volume of distribution, V_T/f_T, where V_T is the tissue volume and f_T is the unbound drug fraction in the tissue (8–10). From this relationship, the modified unbound tissue-to-plasma concentration ratio (K_{pf}) is expressed by

$$K_{pf} = \frac{K_p}{f_p} \tag{1}$$

The relationship in the partition coefficients between two systems of organic solvent (20) leads to Eq. (2):

$$K_{pf} = \alpha \cdot P^{\beta} \tag{2}$$

where β is the slope of the logarithmic plot and α is the value of K_{pf} when P is 1.

This relationship was compared by our present method. A diagrammatic representation of an anatomical compartment in an organism is presented in Fig. 1.

In estimating the K_p of a drug in a compartment under our hypothesis, the following assumptions were made.

(1) In tissue, there are some kinds of lipophilic compartments into which the drug dissolves.
(2) The variations in the distribution in tissue depend on the amount of lipophilic compartment present and on the differences in binding parameters among tissues and species being negligible.
(3) Only the nonionized form which is free from plasma binding can diffuse through the plasma membrane, and at equilibrium the concentration of the nonionized species is the same on both sides of the membrane.
(4) The nonionized form in intracellular fluid is equal to the drug dissolved in the lipophilic compartment.

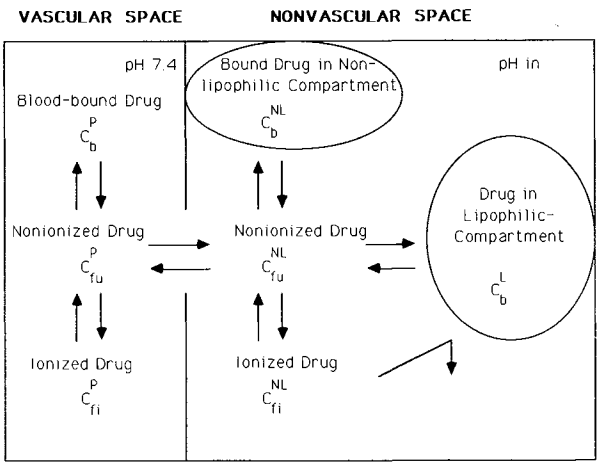

Fig. 1. Diagrammatic representation of the tissue distribution of basic drugs.

Under these assumptions regarding the distribution of drug in the organs, Eq. (3) can be derived to estimate K_p:

$$K_p = \frac{(C_b^L + C_b^{NL} + C_{f_u}^{NL} + C_{f_i}^{NL})}{(C_b^P + C_{f_u}^P + C_{f_i}^P)} \tag{3}$$

where C^L, C^{NL}, and C^P are the drug concentrations in the tissue lipophilic compartment, nonlipophilic compartment, and plasma, respectively. Subscripts b, f_u, and f_i represent, respectively, the bound drug, the nonionized form of the drug free from binding, and the ionized form free from binding. Appropriate rearrangement of Eq. (3) gives

Table II. Tissue-to-Plasma Partition Coefficients (K_p) of Basic Drugs for Various Tissues of Rabbits[a]

			K_p[b]							
Key	log P_{app}	f_p	Lung	Brain	Heart	Gut	Muscle	Adipose	Skin	Bone
1	2.18	0.40	32.1	5.1	6.4	4.3	6.4	2.5	5.2	4.5
2	2.21	0.17	1.8	2.1	1.4	2.2	1.7	2.3	1.6	1.2
3	2.69	0.23	53.5	8.2	14.3	10.8	7.2	27.6	6.2	5.4
4[c]	2.83	0.39	131.0	25.7	34.4	22.5	8.5	120.0	9.9	5.2
5	2.99	0.091	8.4	3.2	6.0	6.7	3.5	12.2	1.6	1.0
6	3.10	0.22	151.4	20.0	35.0	32.9	15.4	132.5	13.5	9.5
7	3.17	0.37	74.3	21.2	22.8	21.5	13.2	76.4	8.1	7.9
8	3.28	0.095	64.0	9.3	14.0	11.1	5.2	40.9	5.4	4.3
9	3.49	0.03	11.0	3.2	2.6	3.6	1.6	5.9	1.4	1.0
10	3.58	0.067	144.3	10.6	40.8	29.2	6.2	86.2	5.6	5.7

[a] Results are given as the mean at 16 hr after the beginning of the infusion studies when the rabbits were sacrificed for tissue sampling. At least three rabbits were used to determine the values. All SE values were within 10% of the mean.
[b] The value of K_p is the ratio of tissue concentration to the arterial blood concentration.
[c] From Refs. 11 and 12.

$$K_p = \frac{f_p \cdot f_{ue}}{f_{ui}} (K_b^{NL} + 1 + f_{ui} \cdot K_b^{L}) \quad (4)$$

where K_b is the concentration ratio of the bound drug to the nonionized drug, f_p is the plasma free fraction, f_{ue} is the fraction of the nonionized form of the free concentration in the extracellular space, and f_{ui} is that fraction in the intracellular space. The pH in the extracellular and intracellular spaces was 7.4 and 7.0 (21), respectively. If the relation $K_b^{NL} \ll (1 + f_{ui} \cdot K_b^{L})$ holds, Eq. (5) is obtained:

$$K_p = f_p \cdot f_{ue} \cdot \left(K_b^{L} + \frac{1}{f_{ui}} \right). \quad (5)$$

The relationship in the partition coefficients between the two systems of organic solvent (20) leads to Eq. (6):

$$K_{pfu} - \frac{1}{f_{ui}} = \alpha \cdot P^{\beta} \quad (6)$$

where K_{pfu} is the ratio of the concentration of unbound and nonionized drug in tissue to the concentration in plasma which was obtained by dividing the K_p value by the product of f_p and f_{ue}. P is the partition coefficient for the drug in each solvent system. α and β are parameters for a selected pair of solvent systems. If the value β is unity, Eq. (6) is equivalent to the relationship between K_{pf} and the apparent octanol–water partition coefficients at pH 7.4. Figure 2 shows the relationship between K_{pfu} and P in various nondisposing organs and tissues.

Table III lists the correlation coefficients calculated with the Eqs. (2) and (6). In many cases the correlation of log K_{pfu} with log P according to Eq. (6) was better than that between K_{pf} and P according to Eq. (2). As shown in Fig. 2, for all tissues, the β value of the equation was close to unity, implying that K_{pfu} is nearly commensurate with the P value. The correlation coefficients are high and they are significant ($P < 0.001$) in all tissues studied. The values of α varied widely from 0.016 to 0.099, and all of them were significant ($P < 0.01$). The correlation coefficients of log K_{pfu} with log P were generally highest in octanol among the organic solvents tested, but all coefficients were highly significant ($P < 0.001$). The evidence shows a high degree of correlation between log K_{pfu} and log P.

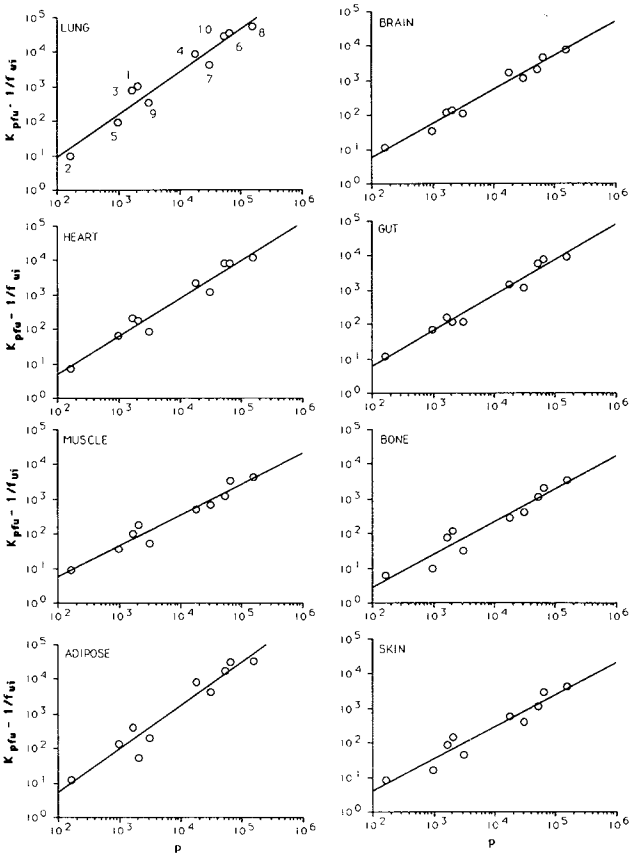

Fig. 2. Relationship in various tissues between tissue-to-plasma partition coefficients of the nonionized form of the drugs (K_{pfu}) and the octanol–water partition coefficients of the nonionized form of the drugs (P). The lines indicate the best fit of Eq. (6).

DISCUSSION

Of the various reports dealing with the relationship of the V_{SS} and the lipophilicity of certain drugs, none has clarified the relationship between K_p in tissues and the lipophilicity of the basic drugs. Weak basic drugs bind not only to albumin but also to β-lipoprotein or α_1-acid glycoprotein (22,23). Since there are interspecies differences in the concentration of binding proteins and the binding affinity of weak basic drugs to plasma proteins, the value of f_p varies widely. In contrast, there appears to be little difference in the tissue distribution of the drugs between animals and man (8–10,12).

By means of a physiologically based pharmacokinetic approach to the distribution of biperiden, one of the basic drugs, we previously confirmed that (i) the K_{pf}'s for each tissue were in good agreement between rats and rabbits (12), (ii) the two major tissues in which biperiden was distributed were fat and muscle (11,12), and (iii) there was a linear relationship between the V_{SS}/BW and the fat volume per lean mass body weight in 4-, 10-, and 50-week-old rats (25). These results indicate little interspecies variation in binding to tissue components. Moreover, lipophilicity is a dominant determining factor in the distribution of basic drugs in the tissues. As listed in Table I, different orders of partition coefficients at pH 7.4 were obtained among octanol, benzene, chloroform, and triorein. The range of partition coefficients was highest in triorein and narrowest in octanol. With octanol, there was a high correlation in each tissue between log K_{pfu} and log P.

Strictly speaking, there are still problems, especially with regard to intracellular pH and transmural potential difference. Recently, intracellular pH in various rat tissues was reported to be lower than 7.0: 6.89 in lung, 6.88 in brain, and 6.82 in skin (24). But only slight changes in K_p accompany these pH differences. Also, the normal pH inside macrophage lysosomes is 4.7–4.8 (25). Theoretically, the concentration of chlorpromazine, which has the highest pK_a among the drugs in this study, may be 200 times higher in lysosome than in the intracellular space. However, since lysosome occupies only 1% (26) of the cell space, the K_{pf} increase may be less than 11% of the total K_{pf} of chlorpromazine.

With basic drugs, is it not necessary to consider the

Table III. Allometric Parameters Describing Tissue Distribution of Basic Drugs in Relation to the Lipophilicity

	Eq. (2) using $P_{octanol}$			Eq. (6) using $P_{octanol}$					
	α	β	r	α	β	r	$P_{triorein}$, r	$P_{benzene}$, r	$P_{chloroform}$, r
Lung	1.518	0.565	0.851	0.031	1.236	0.969	0.917	0.915	0.868
Brain	3.157	0.312	0.787	0.062	0.984	0.987	0.902	0.924	0.876
Heart	1.678	0.422	0.780	0.032	1.098	0.976	0.920	0.926	0.894
Gut	3.002	0.346	0.691	0.058	1.020	0.985	0.927	0.931	0.902
Muscle	4.928	0.221	0.713	0.099	0.889	0.973	0.861	0.892	0.836
Adipose	0.915	0.573	0.769	0.016	1.255	0.965	0.942	0.935	0.939
Skin	2.997	0.256	0.789	0.058	0.927	0.964	0.857	0.868	0.810
Bone	1.975	0.273	0.776	0.036	0.947	0.960	0.847	0.851	0.792

difference in potential across plasma membranes? Although some drugs induce changes in the transmural potential difference associated with the uptake into the cell (27), in the steady state that potential may not affect the accumulation of the drugs in the cell. A possible explanation is that the potential is controlled mainly by Na^+ ion flux and/or H^+ ion flux, which is transported through the plasma membrane faster than the drugs.

In conclusion, there was a good correlation between log K_{pfu} and log P; the prediction of the K_p according to Fig. 1 was successful for most of the drugs studied (Fig. 2).

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NOMENCLATURE

APC	Organic solvent–water partition coefficient of drug at pH 7.4
f_p	Drug unbound fraction in plasma
f_T	Drug unbound fraction in tissue
f_u	Drug nonionized fraction of unbound drug at pH 7.4
f_{ue}	Fraction of nonionized form of free concentration in extracellular space
f_{ui}	Fraction of nonionized form of free concentration in intracellular space
K_p	Tissue-to-plasma concentration ratio at steady state
K_{pf}	Tissue-to-plasma concentration ratio of free drug (K_p divided by f_p)
K_{pfu}	Ratio of the concentration of a nonionized and unbound drug in tissue to the concentration in plasma (K_{pf} divided by f_u)
P	Octanol–water partition coefficient of nonionized form of drug
V_T	Tissue anatomical volume

Superscripts

NL	Intracellular nonlipophilic compartment
L	Intracellular lipophilic compartment
p	Plasma

Subscripts

b	Bound drug
fi	Ionized form of drug free from binding
fu	Nonionized form of drug free from binding

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