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Binding of Drugs to Human Skin: Influencing Factors and the Role of Tissue Lipids

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Abstract—For a series of ten drugs with different physicochemical properties, binding to human skin (epidermis and corium) was determined. Epidermis was obtained by suction blistering, and corium was sliced with a microtome (0·2 mm). Binding experiments were performed in dialysis chambers, containing labelled drug solutions. All drugs investigated were bound to epidermis and corium. With one exception, epidermal drug binding was significantly higher than corial binding. Nevertheless, a good correlation between binding of drugs to both skin fractions could be found. In a range from 10^{-7} to 10^{-3} mol L^{-1} binding of drugs to both skin fractions is linear and not saturable. A good correlation was found between binding and lipophilicity of drugs, determined as the partition coefficients between an organic phase (octanol or heptane) and phosphate buffer of pH 7·0. The results show that binding to epidermis and corium is not saturable and depends on lipophilicity of drugs, indicating unspecific binding. Further binding experiments were performed with lipid-depleted tissue. Since drug binding to lipid-depleted samples and control samples differ only to a moderate extent, it is suggested, that tissue lipids play a marginal role on drug binding. Hence, drugs are bound to human skin by other components like proteins.

For several decades, investigations on drug protein binding were restricted to plasma and its proteins. Since interstitial and intracellular tissue volume is about 13 times greater than plasma volume (Gibaldi & McNamara 1978) and 98 percent of all proteins are located extravascularly (Bickel 1985), a number of authors have pointed out that tissue binding may be of greater influence on distribution and pharmacokinetics of drugs than plasma binding. Hence investigations focused on drug tissue binding and its consequences on pharmacokinetics. Since human skin accounts for about 10% of body weight, it may play an important role in drug distribution. In spite of this possible importance on drug distribution, little information about binding properties of skin has been available hitherto. Among those investigations, binding to skin was only determined for a small number of drugs and in view of topical application (Menczel & Maibach 1972; Baker et al 1977; Dalvi & Zatz 1982; Markantonis et al 1986). Therefore we have measured the binding of ten drugs, representing a broad range of physicochemical properties (degree of ionization, lipophilicity and charge) as shown in Table 1. Since epidermis and corium differ in morphological properties and chemical composition, we determined drug binding to both skin fractions separately. Additional experiments were performed to investigate the factors influencing drug binding and to estimate the role of tissue lipids on drug binding.

Material and Methods

Tissue samples

Human thigh skin was obtained at the autopsy of 27 persons, killed in accidents. The samples were immediately frozen at −30 °C. The epidermal fraction was obtained by the suction blister method of Kiistala (1968) using a laboratory apparatus. From the remaining corium, slices of a thickness of 0·2 mm were prepared with a microtome (Fa. Jung, Heidelberg, FRG).

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Drugs

The non-labelled drugs used were: clonidine HCl (Boehringer Ingelheim, FRG); diazepam and isotretinoin (Hoffmann-La Roche, Grenzach-Wyhlen, FRG); imipramine HCl (Geigy, Wehr, FRG); ketoconazole (Janssen, Neuss, FRG); phenobarbital(one) Na, (Bayer, Leverkusen, FRG); phenytoin (Nordmark, Uetersen, FRG); propranolol HCl (ICI, Plankstadt, FRG); salicylic acid (Merck, Darmstadt, FRG); warfarin Na (Merrell, Rüsselsheim, FRG).

The labelled drugs were obtained from Amersham-Buchler (Braunschweig, FRG) {[^{3}H]clonidine (20 Ci $mmol^{-1}$), [^{14}C]diazepam (0·057 Ci $mmol^{-1}$), [^{3}H]imipramine (23 Ci $mmol^{-1}$), [^{14}C]phenytoin (0·055 Ci $mmol^{-1}$), [^{3}H]propranolol (22 Ci $mmol^{-1}$), [^{14}C]warfarin (0·049 Ci $mmol^{-1}$)}, Hoffmann-La Roche (Basel, Switzerland) {[^{14}C]isotretinoin (0·0009 Ci $mmol^{-1}$)}, New England Nuclear (Dreieich, FRG) [^{3}H]phenobarbital (8·1 Ci $mmol^{-1}$), [^{14}C]salicylic acid (0·052 Ci $mmol^{-1}$)] and Janssen Pharmaceutica (Beerse, Belgium) {[^{3}H]ketoconazole (0·44 Ci $mmol^{-1}$)}.

Binding experiments

About 25 mg epidermis or corium were incubated separately in dialysis chambers in a buffered drug solution (1·8 mL) at pH 7·0. The drug solution contained labelled as well as non-labelled drug at a final concentration from 10^{-7} to 10^{-3} mol L^{-1}. Equilibrium dialysis was performed with a Dianorm apparatus (Diachema, Birmentorf-Zürich, Switzerland) at 4 °C. After equilibrium had been obtained (16 h), the free drug concentration was determined. The water content of each tissue sample was assayed by drying it at 105 °C to constant weight. The total drug amount in tissue was determined by combustion of the specimens in a Packard

Table 1. Physicochemical properties of the drugs tested (these data to the drug not its salt).

Drug	pK_a	% ionization (ph 7·0)	Partition coefficient P (pH 7·0) Octanol	Heptane
Clonidine	8·25[a]	94·67	3·0	$5·8·10^{-3}$
Diazepam	3·31[a]	0·02	720·0	91·5
Imipramine	9·40[a]	99·60	146·0	47·3
Isotretinoin	3·80[b]	99·73	631·7	228·1
Ketoconazole	2·9; 6·51[b]	24·44	5310·5	4·8
Phenobarbital	7·30[a]	33·38	21·0	$5·0·10^{-3}$
Phenytoin	8·33[a]	4·46	289·0	$7·4·10^{-3}$
Propranolol	9·50[a]	99·68	5·4	$4·6·10^{-2}$
Salicylic acid	3·00[a]	99·99	$2·10^{-2}$	$1·8·10^{-4}$
Warfarin	5·00[c]	99·01	28·8	$6·4·10^{-3}$

pK_a values: a) Herzfeldt (1980), b) producer's data, c) Martindale (1982).

sample oxidizer (model #305). Drugs were assayed by liquid scintillation counting in Bray's solution or in a xylene scintillation cocktail, respectively, with a Packard scintillation counter 3385. Assuming that the unbound drug distributes in tissue water, the drug bound was calculated as the difference between total amount of the drug in tissue and the product of free concentration and the volume of tissue water. Binding is expressed as percentage bound or as the ratio bound fraction divided by the free fraction (B/F) which is essentially analogous to a partition coefficient.

Removal of lipids from tissue samples

Before removal of the lipids, epidermis and corium were lyophilized. Thereafter each skin fraction was shaken for 4 h at room temperature (20 °C) in a flask, containing 15 mL of hexane or chloroform–methanol (2:1 v/v) respectively. The amount of extracted lipids was determined by the sulpho-phospho-vanillin reaction, using a commercial standard (Merck, Darmstadt, FRG). Remaining organic solvent was removed in a desiccator at −600 mm Hg. Treated samples and untreated control samples were rehydrated in phosphate buffer (pH 7·0, 0·125 mol L^{-1}) and binding experiments were carried out as described above.

Partition coefficients

The partition coefficients were determined at room temperature between n-octanol or n-heptane and phosphate buffer (pH 7·0, 0·125 mol L^{-1}) according to the method of Leo et al (1971).

Statistics

Results are expressed as the mean ± s.e.m. The statistical significance of observed difference was assessed using Student's *t*-test. A correlation between two parameters was calculated by linear regression. Statistical significance was claimed when $P < 0·05$.

Results

All drugs tested were bound to epidermis and corium to a certain extent. The initial drug concentration in these experiments was 10^{-6} mol L^{-1}. The results are shown in Table 2. For epidermis the fraction bound ranges from

Table 2. Binding of drugs to human epidermis and corium at an initial drug concentration of 10^{-6} mol L^{-1}. Binding is given as percentage bound (% B) and as the ratio B/F (n = 8 – 12).

Drug	Epidermis % B ± s.e.m.	Epidermis B/F	Corium % B ± s.e.m.	Corium B/F
Clonidine	68·46 ± 2·8	2·25	32·01 ± 3·03	0·49
Diazepam	93·73 ± 0·97	18·56	85·88 ± 2·6	9·35
Imipramine	94·52 ± 0·44	18·45	83·09 ± 0·98	5·09
Isotretinoin	99·51 ± 0·05	225·52	97·89 ± 0·36	55·56
Ketoconazole	98·24 ± 0·27	64·29	94·04 ± 0·59	17·02
Phenobarbital	62·27 ± 1·19	1·67	46·52 ± 1·72	0·88
Phenytoin	90·43 ± 0·72	10·66	84·34 ± 1·08	5·7
Propranolol	91·81 ± 0·58	12·02	75·63 ± 0·96	3·18
Salicylic acid	40·64 ± 5·04	0·77	46·01 ± 2·99	0·91
Warfarin	83·51 ± 1·38	5·35	75·45 ± 1·03	3·11

40·64% (salicylic acid) to 99·51% (isotretinoin) and for C from 32·01% (clonidine) to 97·89% (isotretinoin). With the exception of salicylic acid, the drugs were bound significantly higher to epidermis than to corium ($P < 0·01$). Nevertheless a significant relation between binding data of epidermis and corium can be found (Fig. 1).

Dependence of binding on drug concentration

For three acidic and three basic drugs it was investigated if binding to E and C depends on the drug concentration. Fig. 2a, b show the extent of drug binding to both skin fractions over a range from 10^{-7} mol L^{-1} to 10^{-3} mol L^{-1} initial drug concentration. Over this broad concentration range the percentage bound remains essentially constant, indicating linear and not saturable drug binding.

Relation between binding and lipophilicity of drugs

As a measure of lipophilicity, use was made of partition coefficients determined with polar octanol (P_{oct}) and non-polar heptane (P_{hep}). Relations between binding of drugs and a partition coefficient may be expressed by the equation

$$\log B/F = a \times \log P + b$$

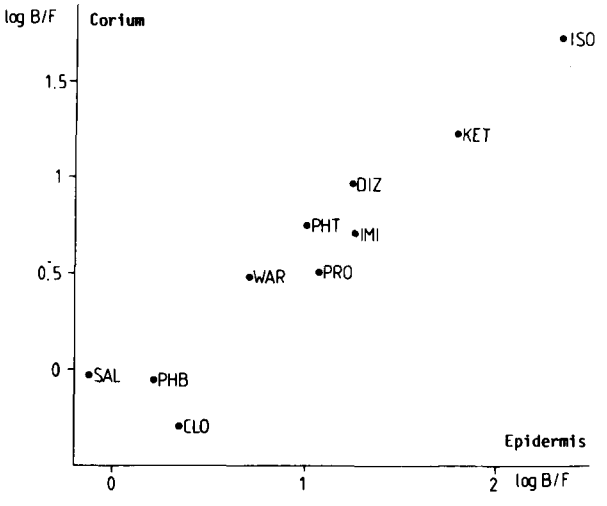

FIG. 1. Relation between binding of drugs to epidermis and corium. Binding data is expressed as log B/F. The correlation is calculated by linear regression. Clonidine = CLO, diazepam = DIZ, imipramine = IMI, isotretinoin = ISO, ketoconazole = KET, propranolol = PRO, salicylic acid = SAL, Warfarin = WAR.

$$r = 0·95;\ P < 0·001$$

$$\log B/F_{epi} = 0·806 \times \log B/F_{cor} - 0·201$$

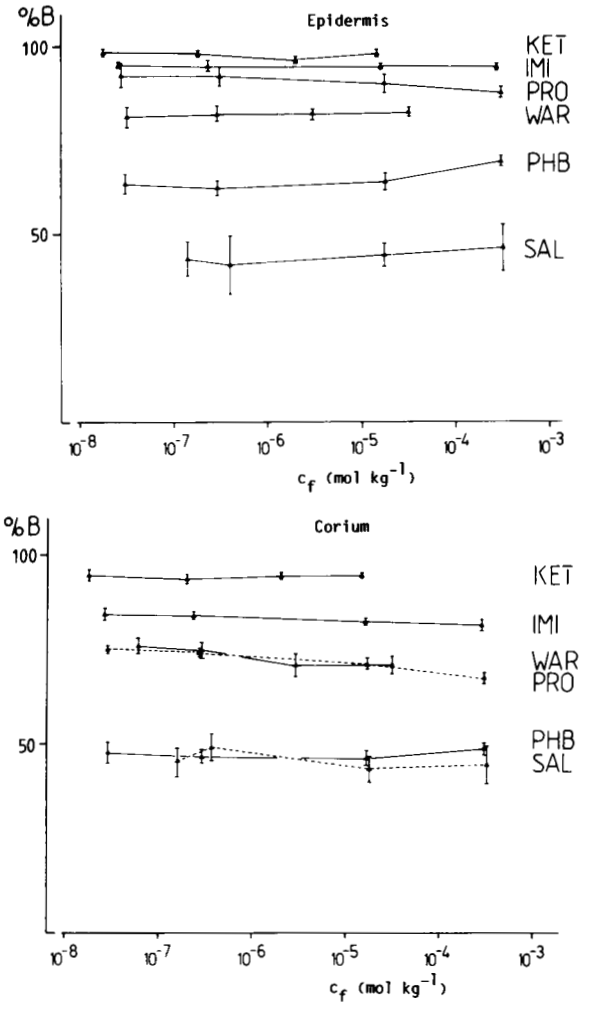

FIG. 2. Dependence of the fraction bound (% B) to epidermis (a) and corium (b) on the free drug concentration in tissue (c_f). Abbreviations as in Fig. 1. (mean ± s.e.m.; n = 4–6.)

where binding is expressed by the fraction B/F, P is the partition coefficient and a and b are constants. Significant correlations can be found between binding of drugs to both skin fractions and the partition coefficients, determined at pH 7·0 (Table 3). As already mentioned, the drugs tested differ in the degree of ionization at pH 7·0. Therefore we calculated the partition coefficient of the non-ionized drug (P'_{oct} and P'_{hep}) via pK_a. Correcting the partition coefficient in this manner, the best correlation coefficients of all were obtained for both skin layers between binding data and the octanol partition coefficient (P'_{oct}).

Binding of drugs to lipid depleted tissue

As shown above, lipophilicity of drugs may be a major prerequisite for extensive drug binding. Since human skin contains considerable amounts of lipids (Adamčič & Fišor-Herman 1967), the question arises whether our data reflect a distribution of the drugs into the tissue lipids or a true binding to tissue components like proteins, for example. To answer this question, tissue lipids were removed by extraction with non-polar hexane and a polar chloroform–methanol mixture (2:1 v/v). Binding data for treated and non-treated specimens are shown in Fig. 3a, b. Removing the epidermal lipids has no influence on binding of phenytoin and isotretinoin. For diazepam a significant decrease in binding results from a treatment with both organic solvents. Removing the corial lipids, we obtained similar results. Here, contrary to epidermis, isotretinoin binding is decreased after the hexane treatment. For ketoconazole the lipid removal with chloroform–methanol enhances binding. Comparing the amounts of lipids removed, the depletion with polar chloroform–methanol seems to be more effective than the hexane treatment (epidermis: 351·4 mg g^{-1} vs 213·4 mg g^{-1}; corium: 306·2 mg g^{-1} vs 165·8 mg g^{-1} dry weight).

Table 3. Correlation coefficient r for the linear regression between log B/F values and partition coefficients, determined with octanol (P_{oct}) and heptane (P_{hep}) at pH 7·0 and the calculated partition coefficients for the non-ionized drug (P'_{oct} and P'_{hep}).

	Epidermis	Corium
P_{oct}	0·81**	0·77**
P_{hep}	0·73*	0·81**
P'_{oct}	0·86**	0·84**
P'_{hep}	0·71*	0·69*

*$P < 0.05$; **$P < 0.01$.

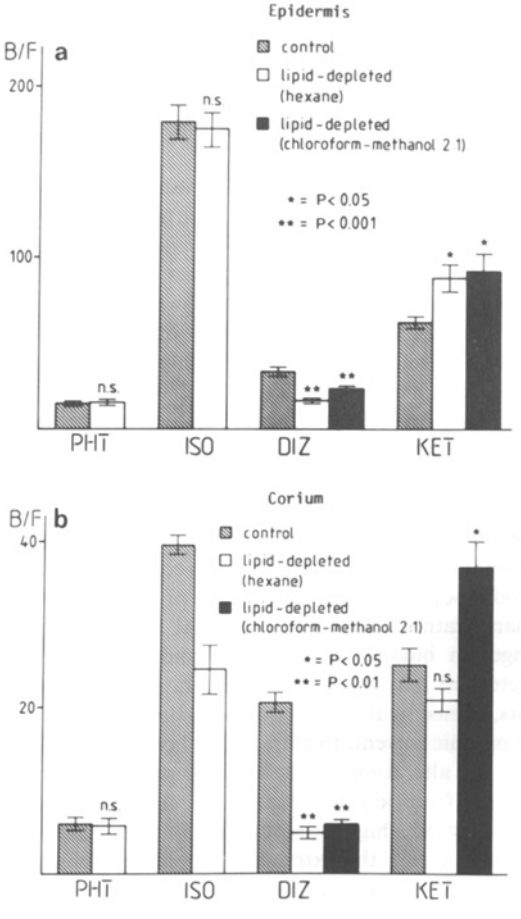

FIG. 3. Binding of drugs to lipid-depleted epidermis (a) and corium (b) Binding is given as the ratio B/F. Abbreviations as in Fig. 1. (mean ± s.e.m.; n = 4–6.)

Discussion

Our aim was not to investigate the binding of drugs during percutaneous penetration. The experiments performed show that human skin may bind drugs as reported for other tissues like muscle (Fichtl & Kurz 1978), brain (Goldberg & Todoroff 1976), liver and lung (Ludden et al 1976). Higher epidermal than corial drug binding has been described in percutaneous penetration studies (Schalla & Schaefer 1982), for retinol (Törmä & Vahlquist 1984) and for benzyl alcohol (Menczel & Maibach 1972). In other studies it was not possible to recognize the different binding to these layers of the skin, because epidermal slices with adherent corium, prepared with a microtome, were used (Menczel et al 1983, 1984). The higher binding of drugs for epidermis cannot find elucidation in the higher lipid content of epidermis, since binding differences between epidermis and corium remain after lipid removal. These findings agree with the in-vivo results from Vahlquist et al (1982), which ascertain that "skin surface lipids contained carotene and retinol, but not in amounts sufficient to contribute to the higher epidermal values".

Binding of drugs to skin is linear. In previous studies a nonsaturable binding capacity of skin or layers of the skin could be found for drugs or other chemicals (Menczel & Maibach 1972; Artuc et al 1980; Menczel et al 1983, 1984). These results may be explained by the great number of binding sites in tissues showing more or less affinity for drugs. For several components of the skin separate binding studies have been performed for melanin (Blois & Taskovich 1969), keratin (Freedman et al 1962), keratinocytes and fibroblasts (Ponec & Kempenaar 1983) and collagen (Eik-Nes et al 1954).

Binding data of epidermis and corium in our studies were compared with the lipophilicity of drugs, measured as the octanol or heptane partition coefficient. In contrast to non-polar heptane, polar octanol has a great capacity for hydrogen bonding and is suggested by a number of authors to be a good model for lipophilicity of drugs in biological systems. Therefore a good relationship between this partition coefficient and binding data of drugs was obtained for human muscle (Fichtl & Kurz 1978), human plasma (Ebling et al 1986) and several tissues (Leo et al 1971). Nevertheless the heptane partition coefficient may be useful to describe binding of drugs to plasma (Kurz & Fichtl 1983). Furthermore, the non-polar cyclohexane partition coefficient, which is comparable to the hexane partition coefficient, seems to be a good model to describe the uptake of several corticoids to human stratum corneum (Saket et al 1984). The observed changes in binding to epidermis and corium after lipid depletement may be due to an alteration of tissue constituents, caused by the extraction procedure. Since hexane is a mild organic solvent, to extract skin lipids (Schmid & Chelf 1976), an alteration of the binding properties should be excluded. Contrary to hexane, the chloroform–methanol mixture extracts higher amounts of lipids, but may damage the tissue during the extraction procedure (Schmid 1973). Nevertheless, regarding the data of our experiments, an essential difference between both organic solvents can be hardly seen. Actually, a removal of the tissue lipids changes the binding of these lipophilic drugs only to a moderate extent. Therefore the major part of a drug may be bound by other tissue constituents such as proteins and glycosaminoglycans. This result agrees well with the findings of binding studies with lipid-depleted muscle (Fichtl et al 1980) and brain tissue (Goldberg & Todoroff 1976). The experiments we have made show that human skin binds drugs to an extent already known from plasma and other tissues. Due to the large volume and the binding properties of human skin, it may be supposed that considerable amounts of a drug may be bound in-vivo, thus influencing distribution and other pharmacokinetic parameters of this drug.

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