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Prediction of skin permeation of highly lipophilic compounds; in vitro model with a modified receptor phase

Alma Dal Pozzo, Enrico Liggeri, Carlo Delucca and Gerardo Calabrese

Istituto di Chimica e Biochimica G. Ronzoni, Via Giuseppe Colombo 81, 20133 Milano (Italy)

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Summary

A skin permeation in vitro model for highly lipophilic compounds was developed, containing bovine serum albumin (BSA) in the receptor phase. Skin permeation rates of three homologous series of compounds varying within a wide range of hydrophobicity were measured. For the more water-insoluble compounds (log $P_{IM} > 3$), it was demonstrated that the modified in vitro model proposed ensures a more efficient collection of the permeant in the receptor phase.

Introduction

The principal requisite for an in vitro model of skin permeation is that the receptor medium should provide an effective sink condition for the permeant. Most in vitro permeation systems contain isotonic aqueous receptor phases; in fact, any other non-physiological fluid, such as organic solvents or surfactant solutions, is likely to damage the skin, or have no general application and make arduous the chemical analysis of the permeating molecule.

Problems arise when highly lipophilic compounds (log $P > 3$) are under study, because of their low tendency to partition into the aqueous receiver medium beneath the skin. The increase in volume of the receptor phase, as in the flow-through cell designs, tends to minimize collection problems, but in many cases lowers the drug concentration below the sensitivity of the analytical method used, unless radiolabelled compounds are employed, which is obviously impractical during the screenings of molecules under preformulation study.

In order to optimize the receptor fluid for lipophilic compounds, a method was developed using diffusion chambers where a physiological solution of albumin was substituted for the simple isotonic aqueous solution. Albumin is present in the intact vascular system with the main function to take up and to transport lipophilic solutes. The protein has a good solubilizing power, because of its binding with lipophilic compounds, the binding is reversible and the protein can be easily removed before analytical determinations.

Other authors had previously noted increased permeation of hexachlorophene (Brown and

Correspondence: A. Dal Pozzo, Istituto di Chimica e Biochimica G. Ronzoni, Via Giuseppe Colombo 81, 20133 Milano, Italy.

Ulsamer, 1975), cortisone and cinnamyl anthranilate (Bronaugh and Stewart, 1984) when BSA was added to the receiving phase.

The aim of our work is to demonstrate that a physiological albumin solution may have general use in permeation models, because the protein can bind any organic lipophilic molecule either specifically or nonspecifically, even though with different capacity, and because the apparent association constants increase with increasing log P of the ligand (Hansch and Church, 1972).

What is more, the binding to albumin, which brings the ligand molecule into the aqueous solution, occurs very rapidly (of the order of milliseconds) when compared to the times required for the normal solubilization processes to reach equilibrium.

Three different homologous series of compounds, covering a wide span of lipophilicity (log P between -1.72 and $+5.96$) and skin permeability (log $\bar{J}$ between -0.66 and -3.78) were employed in this study, and the concentration of the permeants in the receiving phase was determined by HPLC.

The compounds under investigation were applied to the epidermis in a pure state, in order to avoid side effects due to vehicles.

Materials and Methods

The three homologous series of compounds used for the experiments are reported in Table 1. They are, respectively, 10 alkyl nicotinates of structure C_6H_4NCOOR (**1a–1l**), four acyl esters of phenoxyethyl alcohol of structure $C_6H_5OCH_2CH_2$-OCOR (**2a–2d**) and seven alkyl carbonates of phenoxyethyl alcohol of structure $C_6H_5OCH_2$-CH_2OCOOR (**3a–3g**).

TABLE 1

Increase in water solubility of compounds of general formula x-CO-R in the presence of bovine serum albumin

Compound	x	R	Aqueous saline solution (mg/ml)	Aqueous saline solution + BSA (mg/ml)	Solubility increase (ratio)
1a	C_5H_4N	$O(CH_2CH_2O)_3H$ [a]			
1b		$OCH_2CH(CH_3)OH$[a]			
1c		$O(CH_2CH_2O)_3CH_3$ [a]			
1d		$OCH_2CH_2OCH_3$ [a]			
1e		OC_2H_5	42	42	1
1f		OC_4H_9	2.40	3.84	1.6
1g		$OCH_2C_6H_5$	0.60	1.96	3
1h		OC_6H_{13}	0.14	1.30	9.3
1i		OC_8H_{17}	0.01	0.84	84
1l		$OC_{10}H_{21}$	0.004	0.71	178
2a	C_6H_5-$O(CH_2)_2O$	CH_3	1.5	2.2	1.5
2b		C_2H_5	0.49	1.0	2
2c		C_5H_{11}	0.0035	0.3	86
2d		C_7H_{15}	0.0008	0.25	312
3a	C_6H_5-$O(CH_2)_2O$	$O(CH_2CH_2O)_3CH_3$	4.48	5.48	1.2
3b		$O(CH_2CH_2O)_2CH_3$	2.82	3.40	1.2
3c		$OCH_2CH_2OCH_3$	0.726	1.265	1.7
3d		OC_2H_5	0.283	0.948	3.3
3e		OC_4H_9	0.028	0.373	13.3
3f		OC_6H_{13}	0.0022	0.283	129
3g		OC_8H_{17}	0.0003	0.151	503

[a] These compounds are freely soluble in water.

TABLE 2

Skin permeation data (log $\bar{J}$) determined in the presence and in the absence of BSA, and isopropylmyristate / water partition coefficient expressed as log P_{IM}

Compound	Log P_{IM}	Log $\bar{J}$ (cm/h)($\times 10^3$) in saline	Log $\bar{J}$ (cm/h)($\times 10^3$) in saline + BSA
1a	−1.72	−2.45 [a]	−2.5
1b	−1.20	−2.14 [a]	−2.10
1c	−0.64	−1.61 [a]	−1.60
1d	0.24	−0.91 [a]	−0.93
1e	0.92	−0.66 [a]	−0.65
1f	2.12	−1.10 [a]	−1.17
1g	2.42	−1.69 [a]	−1.75
1h	3.32	−1.91 [a]	−1.79
1i	4.52	−3.19	−2.4
1l	5.72	nd [b]	−2.82
2a	2.05	−1.45	−1.46
2b	2.64	−1.62	−1.65
2c	4.34	−3.17	−2.5
2d	5.54	nd [b]	−3.09
3a	1.05	−2.16	
3b	1.43	−1.65	−1.6
3c	1.86	−1.95	
3d	2.69	−1.93	−1.98
3e	3.75	−2.6	−2.32
3f	4.86	−3.78	−2.95
3g	5.96	nd [b]	−3.53

[a] Data from Dal Pozzo et al. (1991).

[b] Not determined because their concentration was below the sensitivity of the analytical method used.

Except for ethyl and benzyl nicotinates, which are commercially available, all other compounds were prepared in our laboratory by common synthetic techniques.

Crystallized and lyophilized BSA was purchased from Sigma.

Solubility measurements

The apparent solubility in aqueous saline solution, with or without albumin, was determined as follows: Aliquots of each compound corresponding to at least 20% in excess as compared to its known saturation concentration, were suspended in several test tubes containing, alternatively, either 5 ml of a solution of 0.06 M phosphate buffer, pH 7.4, with 0.9% sodium chloride, or the same supplemented with 4.5% of BSA. The test tubes were shaken at 25 °C for 1 h, then centrifuged at 5000 rpm for 1 h.

1 ml of the aqueous phase was added to 5 ml of a mixture of acetonitrile-methanol 80 : 20, supplemented with a suitable internal standard, stirred for 30 s and centrifuged again.

Concentrations of the sample compounds were determined in the supernatant by HPLC. For compounds with solubility less than 2 μg/ml it was necessary to use a modified technique: after centrifugation, the supernatant solution was filtered repeatedly through filter paper, discarding the first few milliliters of the filtrate, in order to avoid errors due to traces of oily suspension, then a measured volume was extracted three times with chloroform (1×10 and 2×5 ml), the organic solvent evaporated under a nitrogen atmosphere and the residue dissolved in the minimum amount of methanol.

This technique cannot be applied in the case of nicotinates which tend to evaporate with the solvent.

Determination of partition coefficients isopropyl myristate / water and skin permeation rates

The experimental procedures employed have been described previously (Dal Pozzo et al., 1991).

Common static diffusion chambers with human epidermis were used, where the receptor phase was isotonic saline solution with or without 4.5% BSA. Determination of the permeants in the receptor phase, at given time intervals, was carried out by HPLC, after denaturation of the protein, when necessary, using the same method as described above.

Partition coefficients were expressed as log P_{IM}. Skin permeation rates were calculated as $\bar{J}$ according to Eqn 1:

$$\bar{J} = J_s / C \quad (1)$$

where J_s is the flux observed under the experimental conditions and C represents the concentration of compound in the donor phase; a concentration of 1000 mg/ml was assumed in this case, because the compounds were applied as pure liquids, disregarding small errors due to the different densities.

Results and Discussion

The solubility data are reported in Table 1.

As expected, the water solubility of all compounds, irrespective of their chemical structure, increased in the presence of BSA, the increase being positively correlated with log P, as shown in Fig. 1.

These results are consistent with the knowledge that the affinity of non-ionized molecules to non-specific hydrophobic sites of the protein is always correlated with their partition coefficients (Helmer et al., 1968).

In Figs 2–4, the logarithms of the skin permeation rates of the three different series of compounds, in the absence or presence of albumin, have been plotted vs log P; the presence of the protein in the receptor fluid caused an evident positive effect on the permeation of the more lipophilic compounds.

It is interesting to note that the solubility of compounds **1f, 1g, 2a, 2b, 3b** and **3d** was increased by BSA, although their permeation rate remained unchanged.

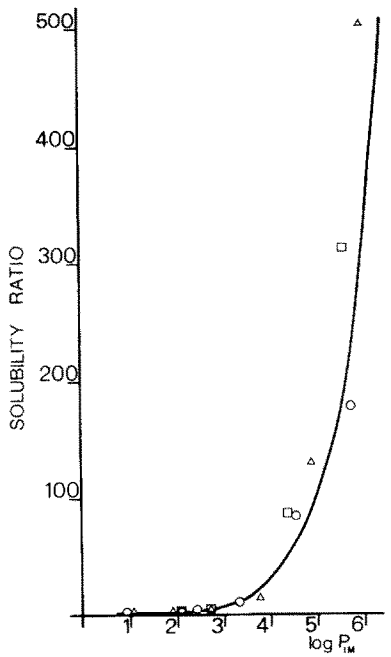

Fig. 1. Increase of water solubility in the presence of BSA, of all compounds studied expressed as the solubility ratio in the presence and absence of the protein, vs log P_{IM}. Compound **1a–1l** (○); **2a–2d** (□); **3a–3g** (△).

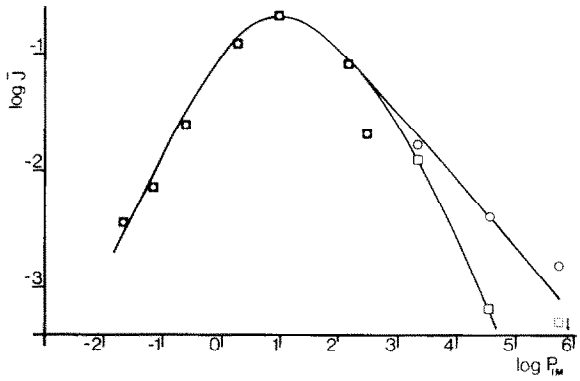

Fig. 2. Skin permeation rates (log $\bar{J}$) vs log P_{IM} of the alkyl nicotinates (**1a–1l**) into buffered saline solution (□) or into the same containing BSA 4.5% (○).

This means that the chamber with simple saline solution as receptor fluid, under the experimental conditions used, can give reliable data for compounds ranging up to log $P = 2$. However, for more lipophilic compounds (log $P > 3$) this system is not able to provide optimal sink conditions.

Another advantage with the use of BSA is that the increase in the concentration of permeant in the receiving solution allows chemical determination of the less soluble compounds, which would otherwise be below the sensitivity of the analytical

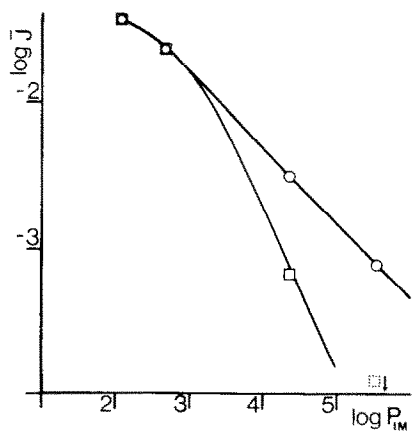

Fig. 3. Skin permeation rates (log $\bar{J}$) vs log P_{IM} of the phenoxyethyl acyl esters (**2a–2d**) into a buffered saline solution (□), or into the same containing BSA 4.5% (○).

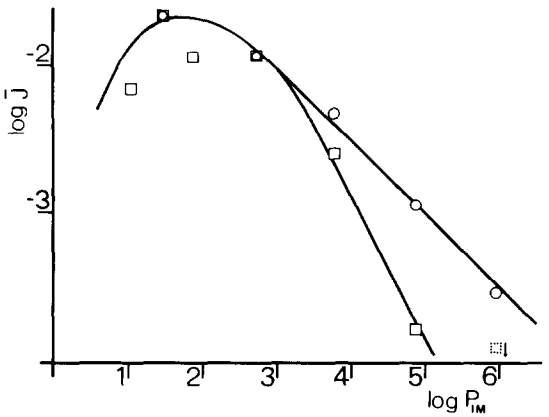

Fig. 4. Skin permeation rates (log $\bar{J}$) vs log P_{IM} of phenoxyethyl alkyl carbonates (**3a–3g**) into a buffered saline solution (□), or into the same containing BSA 4.5% (○). Compound **3c** (log P_{IM} 1.86) does not fit the drawn profile, but this is very probably due to viscosity reasons, because **3c** is the only solid compound at the temperature of the experiment and was applied to the skin in acetone solution, followed by evaporation of the solvent.

method used. This was the case for compounds **1l**, **2d** and **3g**.

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