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Report

Enhanced *in Vitro* Skin Permeation of Cationic Drugs

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The lipophilicity of cationic drugs can be increased by forming ion pairs with the carboxylate anion of fatty acids. Transport of cations across an isopropyl myristate (IPM) membrane was facilitated in the presence of oleic acid and lauric acid, providing an appropriate pH gradient existed. Enhancement of *in vitro* skin permeation of various drugs, in the presence of fatty acids, was shown to be more dramatic with the slow-permeating neutral caffeine and anionic salicylate. Since both molecules are unable to form ion pairs it is probable that the fatty acids are capable of exerting a disruptive influence on the skin. The cationic drugs appeared to traverse excised human skin more rapidly than predicted by the model membrane data. This may be due to ion pairing with free fatty acids or other anionic groups within the skin. Consequently, the enhancing ability of fatty acids was less marked for neutral or anionic permeants.

KEY WORDS: skin permeation; β-blockers; fatty acids; caffeine; naphazoline; sodium salicylate; isopropyl myristate; rotating diffusion cell.

INTRODUCTION

The objective of the present work was to investigate the physicochemical aspects of enhanced skin permeation of cationic drugs. This is an area of particular importance since many drugs contain a basic nitrogen atom, which may be positively charged at physiological pH.

Simply, the skin can be considered as a trilaminate structure, consisting of the outer, dense keratinized layer known as the stratum corneum, the underlying viable hydrophilic epidermis, and the dermis, which supports the vasculature. For charged hydrophilic molecules the lipophilic stratum corneum presents the greatest barrier to skin permeation. This layer can be thought of as a "bricks and mortar" structure (1). The bricks represent the keratinocytes, while the mortar is composed of a diverse mixture of lipids including sphingolipids, fatty acids, free sterols, triglycerides, and nonpolar lipids (2). Molecules can permeate through the stratum corneum through either transcellular, intracellular, or shunt routes (skin appendages). However, the intercellular route has been shown to be the more dominant pathway for diffusion of small organic molecules (3).

The amphiphilic nature of the skin dictates that its permeability will be highly dependent on the physicochemical properties of the penetrant. The partition properties of the molecule must be balanced for effective permeation, as illustrated in Fig. 1. For hydrophilic penetrants (region A) partitioning into the stratum corneum is rate determining; however, at higher lipophilicities (region B) partitioning out of the stratum corneum into the viable epidermis becomes important.

As a means of increasing the relative lipophilicity of cations to an optimal log P for skin permeability we have investigated the use of ion pairs. Here the skin is impregnated with fatty acids, which at pH 7.4 will be negatively charged. Oleic acid and lauric acid were selected as model anionic carriers, as they appear to be well tolerated by the skin (4) and have previously been used to enhance the skin permeation of polar and nonpolar molecules (5,6). The effects of the fatty acids on the partitioning behavior and permeation across model and human skin membranes were studied with a series of model cationic, anionic, and neutral drugs.

CHOICE OF MODEL DRUGS

A series of structurally related water soluble β-blockers was chosen as model cationic drugs. The lipophilicity of these has been characterized previously according to their octanol/water distribution coefficients and found to fall into three groups: lipophilic, hydrophilic, and intermediate in behavior (7). Representative drugs from each of these categories were selected: bupranolol and propranolol from the first group, atenolol from the second, and oxprenolol and metoprolol from the last. The structures and some physicochemical parameters of these are given in Table I. The β-blockers, particularly the more lipophilic members, are subject to extensive first-pass hepatic metabolism, while atenolol also has a low oral bioavailability due to incomplete absorption. Consequently, transdermal administration of such potent drugs, which require continuous well-controlled delivery, would be advantageous. In addition, the short plasma half-lives of the

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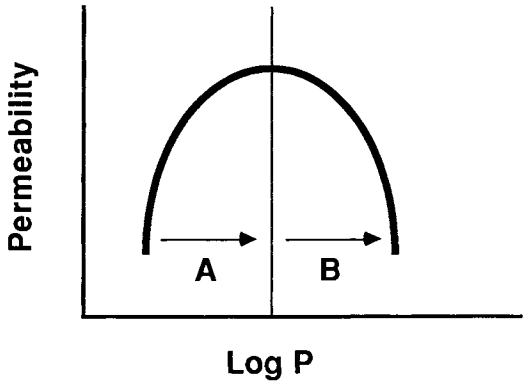


Fig. 1. Effect of oil/water partitioning (*P*) on solute skin permeability.

β-blockers enables steady-state conditions to be achieved rapidly. Caffeine and sodium salicylate were selected as model neutral and anionic penetrants, respectively. Naphazoline was chosen as a further cationic drug since it is more basic than the β-blockers.

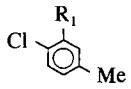
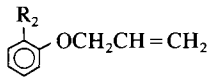
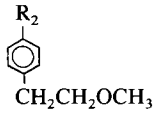
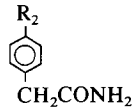
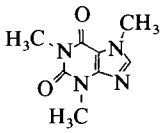
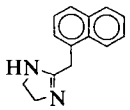
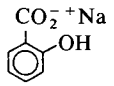
MATERIALS AND METHODS

Bupranolol was obtained from Schwarz GmbH (Monheim, Germany). All other materials and methods used to determine isopropyl myristate (IPM)/water distribution coefficients (K_d), model membrane permeability coefficients (k_i), and skin permeability (k_p) of the model permeants are outlined in previous papers (9,10). Dermatomed skin (310-μm thickness) was used as a membrane for studying the diffusion of bupranolol. This molecule is the least water soluble of all the model permeants, and as a result thinner skin sections were used to provide a better model for *in vivo* permeation (11). Dermatomed skin was prepared using a Davies duplex 7, Gold series, electric skin dermatome (Model GD103, C. F. Thackray). High-performance liquid chromatography (HPLC) was used to analyze permeants within the receptor phase of the skin diffusion cells (10). Table II outlines the various other chromatographic parameters.

RESULTS AND DISCUSSION

Table III shows the effect of oleic and lauric acids on the

Table I. Physicochemical and Pharmacological Data of Model Permeants (8)

Chemical name	Structural formula[a]	Aqueous solubility (*M*)[b]	pK_a	Plasma $\frac{1}{2}$-life (hr)
Bupranolol[c]		0.02[d]	9.6	2–6
Propranolol		0.17	9.5	3–4
Oxprenolol		3.32	9.5	1–3
Metoprolol		Very	9.7	2–7
Atenolol		Very	9.6	4–14
Caffeine		0.09	<1.0	—
Naphazoline		0.68	10.9	—
Sodium salicylate		6.25	3.0	—

[a] R_1 = $OCH_2CH(OH)CH_2NHC(CH_3)_3$; R_2 = $OCH_2CH(OH)CH_2NHCH(CH_3)_2$.
[b] Concentration of saturated aqueous solution of salt.
[c] Schwarz GmbH.
[d] pH 5.5.

Table II. Reverse-Phase Chromatographic Conditions Required to Analyze Skin Permeants, Where the Mobile Phase Consists of 5 m*M* Heptane Sulfonic Acid in 5 ml Glacial Acetic Acid, *A* ml Methanol, and *B* ml Previously Boiled Distilled Water

Drug molecule	Mobile phase		Detector Wavelength (nm)	Flow rate (ml/min)	Retention time (min)
	A	*B*			
Bupranolol	830	165	275	1.5	4.2
Propranolol	830	165	289	1.5	5.4
Oxprenolol	750	245	273	1.5	4.8
Metoprolol	700	295	274	1.5	4.1
Atenolol	420	575	273	1.5	5.0
Naphazoline	950	45	280	2.5	3.7
Caffeine	400	595	273	1.0	4.0
Sodium salicylate	342 ml isopropyl alcohol, 10 ml formic acid to 1 L with 0.1 *M* potassium dihydrogen orthophosphate (aq)		298	1.0	4.5

partitioning (K_d), transport across an isopropyl myristate membrane (k_i), and skin permeability (k_p) for all model compounds (9). The transfer of cationic molecules across an IPM membrane can be facilitated by the fatty acids. Figure 2 shows the relationship between the model membrane permeability coefficient (k_i) and the distribution coefficient (K_d) for the model permeants. The figure shows that transfer rates across IPM membranes are increased with permeant lipophilicity. However, for cationic permeants, traversing the IPM membrane loaded with the fatty acids, the increase in k_i is less than expected. This may be as a result of the formation of a bulky, slow-diffusing, ion pair and/or a decrease in the rate of partitioning out of the membrane into the receptor phase.

From the skin permeation experiments it appears that the enhancers are selective in their action, having the greatest effect upon the more hydrophilic compounds that permeate the skin slowly (Fig. 3; an enhancement ratio, *E*, has been included, to represent the ratio of the permeabilities of pretreated skin to those across control skin), particularly salicylate and caffeine, which are anionic and uncharged respectively. Since both molecules are not expected to form ion pairs it is possible that the fatty acids exert their enhancing effect through a perturbation of the stratum corneum. This conclusion was further supported by *in vivo* (10) and *in vitro* (12) data. Thermal analysis (12) of skin has suggested that the nonlinear structure of oleic acid can disrupt the stratum corneum intercellular lipid bilayers. Subsequent lipid

Table III. The Effect of Oleic Acid on the Distribution Coefficient, Model Membrane Permeability, and Skin Permeability of Model Permeants

Chemical name	K_d		k_i (μmsec^{-1})		k_p ($\times 10^{-10}$ msec^{-1})*		
	pH 7.4	pH 7.4 + oleic acid	IPM	IPM/0.1 *M* oleic acid	Control	+ Oleic acid	+ Lauric acid
Bupranolol (BP)	2.89 ± 0.34[a]	245.6 ± 50.5[a,b]	6.11 ± 0.81[c]	14.36 ± 0.27[c]	60.0 ± 8.1	49.7 ± 7.8	57.8 ± 8.6**
Propranolol (PP)	1.87 ± 0.09[c]	51.63 ± 5.11	3.92 ± 0.23	6.92 ± 0.78	34.2 ± 10.6	48.6 ± 20.6**	50.6 ± 19.7**
Oxprenolol (OX)	0.18 ± 0.02	5.67 ± 0.62	0.22 ± 0.02	0.94 ± 0.11	48.6 ± 11.1	83.6 ± 11.4	65.0 ± 20.0
Metoprolol (MT)	0.05 ± 0.01	1.87 ± 0.21	0.12 ± 0.02	0.45 ± 0.05	23.3 ± 7.2	46.4 ± 10.6	38.9 ± 7.8
Atenolol (AT)	ND		ND		6.4 ± 2.8	15.0 ± 5.8	11.9 ± 3.3
Caffeine (CF)	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.13 ± 0.02	7.2 ± 4.2	100.6 ± 31.9	77.5 ± 16.4
			k_i (nmsec^{-1})				
Naphazoline (NZ)	0.02 ± 0.01	0.36 ± 0.04	6.7 ± 0.8	185.6 ± 18.9	9.2 ± 0.8	58.9 ± 6.9	72.2 ± 13.6
Sodium salicylate (SS)	ND		ND		0.6 ± 0.2	31.4 ± 7.8	25.0 ± 5.0

[a] Aqueous phase consisted of 0.5% ethanol in phosphate-citrate buffer.
[b] Organic phase contained 2 ml of 0.2 *M* fatty acid in IPM.
[c] Donor phase contained 3.4 m*M* drug in 3.4% ethanol in pH 8.0 phosphate buffer.
* $P < 0.05$ two-way ANOVA between pretreated and control sites.
** $P > 0.05$ two-way ANOVA between pretreated and control sites.

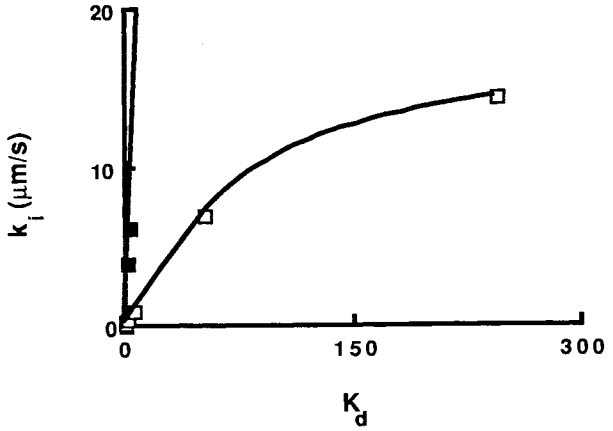


Fig. 2. The effect of lipophilicity (K_d) on the permeability (k_i) of model permeants across an isopropyl myristate (■) and 0.1 *M* oleic acid in isopropyl myristate membrane (□).

fluidisation may result in an increased diffusion rate to permeating molecules. The possible reason for the small enhancement of naphazoline and the β-blockers (with the exception of propranolol and bupranolol) by the fatty acids may be a result of their relatively high skin permeation, i.e., the intercellular lipids are presenting less of a barrier to skin diffusion. Consequently, attempts to reduce this barrier by pretreatment with penetration enhancers will result in a less-noticeable increase in skin permeation. Figure 4a shows the relationship between permeability coefficients of skin (k_p) and IPM model membranes (k_i) within the rotating diffusion cell. The data points are from this study and previous works where the permeability of the IPM membrane to a diverse set of penetrants was determined (13). Linear regression analysis of the data in Ref. 13 has been used to produce a straight line of the data. The values excluding those for isoquinoline and nicotine, since these compounds are known to have abnormally high skin permeability coefficients, yielded the best-fit line drawn through the data (Fig. 4a), which is described by

$$\log k_p = 1.06 \log k_i - 3.09; \quad r = 0.925 \qquad (1)$$

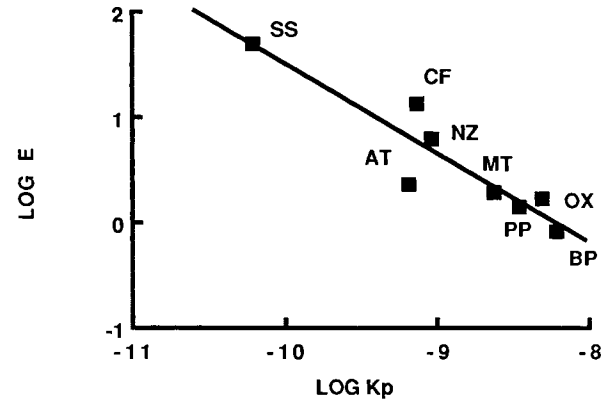


Fig 3. Correlation of skin permeability (k_p) with the penetration enhancement effect of oleic acid, where *E* is the mean enhancement ratio defined as $E = k_p$ (pretreated skin)/k_p (control). Abbreviations for the model permeants are given in Table III.

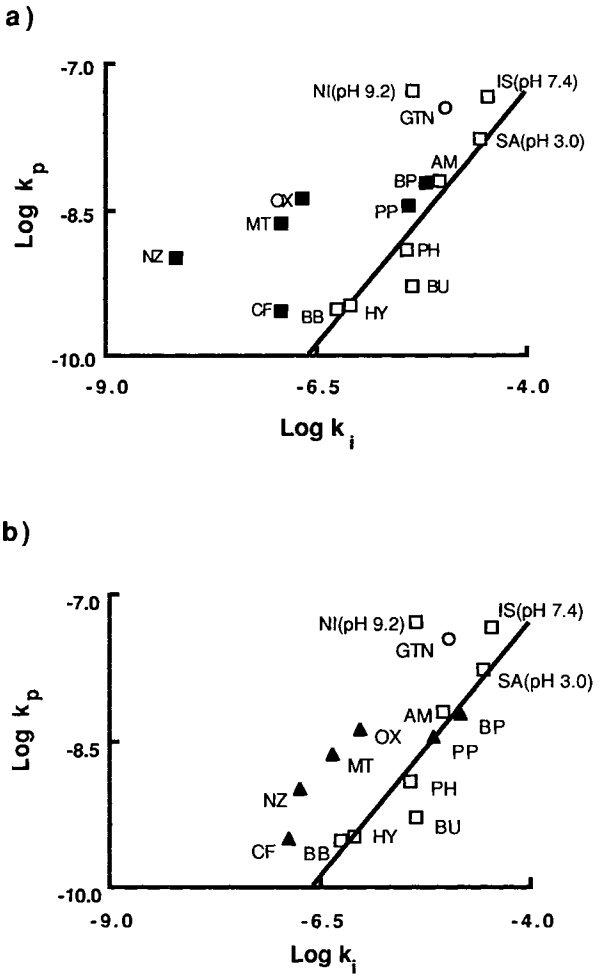


Fig. 4. Relationship between permeability across excised human skin (log k_p) and across (a) an isopropyl myristate membrane with the rotating diffusion cell (log k_i) and (b) 0.1 *M* oleic acid in isopropyl myristate membrane. (Lauric acid gave similar results.) Data points (□) from Ref. 13 (donor phase, pH 5.0 phosphate buffer except where stated) and (■, ▲) from Table III. The values of k_p for GTN (donor phase, deionized water) and BP were measured across dermatomed skin (310 μm). AM, amylbarbitone; BB, barbitone; BP, bupranolol; BU, butobarbitone; CF, Caffeine; GTN, glyceryl trinitrate; HY, hydrocortisone; IS, isoquinoline; MT, metoprolol; NI, nicotine; NZ, naphazoline; OX, oxprenolol; PH, phenobarbitone; PP, propranolol; SA, salicylic acid.

The gradient (1.06) of the line indicates that k_p and k_i are closely related, although the permeability through the IPM membrane was approximately 1000 times greater than through human skin. When the values for the β-blockers, caffeine, and naphazoline were added to the data in Ref. 13, the degree of correlation between k_p and k_i became less. This is reflected by Eq. (2):

$$\log k_p = 0.242 \log k_i - 7.28; \quad r = 0.450 \qquad (2)$$

Examination of Fig. 4a suggests that the IPM membrane does not accurately reflect the high permeability of the skin to the more hydrophilic β-blockers (oxprenolol, metoprolol, and atenolol, not illustrated since k_i was too low to be measured), naphazoline, nicotine, isoquinoline, and glyceryl

trinitrate. For the first four cationic compounds a possible explanation for this anomaly may be due to their intrinsic charge; the β-blockers and naphazoline are all positively charged species at the experimental pH. Fatty acids account for approximately 20% of the stratum corneum lipids (2). Consequently, there is a possibility that each cation may form ion pairs with these endogenous free fatty acids, thus facilitating its own skin permeation. It has been reported that the skin behaves as a negatively charged permselective membrane allowing through cationic permeants (14). The high skin permeation of the charged nicotine, isoquinoline, and glyceryl trinitrate may be due to their amphiphilic structure. They may have structural features which confirm intrinsic penetration enhancement properties upon the molecule. The membrane of the rotating diffusion cell consists of a homogeneous IPM layer, in marked contrast to the heterogeneous composition of the stratum corneum; another possible explanation for the variation seen. The correlation for the β-blockers can be improved [Eq. (3)] if the permeability data from excised human skin are compared with those from IPM membranes treated with oleic or lauric acids (Fig. 4b). This indicates that the presence of endogenous fatty acids may be an important factor in the case of cationic transport.

$$\log k_p = 0.687 \log k_i - 4.80; \qquad r = 0.774 \qquad (3)$$

The skin permeation of propranolol and bupranolol appears to be reasonably well predicted by the IPM membrane (Fig. 4a). This may be because the increased skin permeation, resulting from ion pairing with the intercellular fatty acids, is counterbalanced by a reduction in the partitioning out of the stratum corneum into the viable epidermis (Fig. 1). For these lipophilic compounds further increases in lipophilicity, resulting from ion pairing, may significantly retard their release from the stratum corneum into the hydrophilic viable epidermis. For bupranolol this may also account for the observed reduction in skin permeation in sites pretreated with oleic acid (Table III).

In conclusion, the results obtained in this paper suggest that fatty acids can be used to promote skin permeation of hydrophilic drugs, which traverse the skin slowly. The ability of the acids to increase the flux of cationic drugs across the skin is possibly by an ion-paired facilitated transport mechanism and lipid fluidization.

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