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Facilitated percutaneous absorption: a comparison and evaluation of two in vitro models

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Summary

Studies have been conducted in a model system for percutaneous absorption, the rotating diffusion cell, using an artificial membrane representative of the epidermal barrier. An ethoxylated amine, Ethomeen S12 (bis-(2-hydroxyethyl)oleylamine), was investigated for its ability to promote the percutaneous absorption of the salicylate anion, by a facilitated transport process, using a pH gradient of 5–7.4 across the membrane to provide the free energy required for the co-transport mechanism. The transport of caffeine, a cationic drug, across the model membrane was not enhanced using this system. In vitro skin studies were conducted to establish a facilitated transport mechanism within the stratum corneum. The transdermal penetration of sodium salicylate was enhanced, but so too was that of caffeine, indicating that this type of compound may reduce the skin barrier by a mechanism independent of observed carrier properties in the model system.

Introduction

Many drugs which may be used either for a local effect or for systemic effect following transdermal delivery are weak acids or bases. At the pHs normally experienced under physiological conditions they will be ionized. This will reduce their ability to partition into the lipophilic outer regions of the stratum corneum and hence minimize their therapeutic potential. In order to deliver such drugs it may be possible to utilize an ion pair mechanism such as that of facilitated transport.

The facilitated transport of anionic drug molecules across artificial lipid membranes representative of the stratum corneum, utilizing long chain tertiary alkanolamines as the carrier molecules has been previously reported (Barker and Hadgraft, 1981; Barker et al., 1984).

The surface of human skin is reported to be slightly acidic (pH 4.2–5.6) and the lower layers are at the physiological pH of 7.4 (Poulsen et al., 1968). A pH gradient therefore exists across the stratum corneum that might provide the free energy difference required to drive a facilitated transport scheme in vivo.

The facilitated transport scheme is illustrated in

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Fig. 1 and can be established in the rotating diffusion cell, an in vitro model for percutaneous absorption. The epidermal barrier is simulated using a cellulose nitrate membrane filter impregnated with isopropyl myristate (IPM), a liquid representative of skin lipids. For the purposes of our investigations the carrier is incorporated within the IPM membrane. At the lower pH of the donor compartment/membrane interface the carrier protonates and combines with anions present in the interfacial region to form electrically neutral ion pairs. The ion pairs can then partition into the bulk lipid phase and diffuse down their own concentration gradient to the opposite interface. In the interfacial region at the higher pH the carrier deprotonates to release the anions. Consequently, the carrier molecule is free to diffuse back to the original interface and repeat its role. Using this mechanism it is possible to transport anions against their own concentration gradient.

In this paper the feasibility of using this scheme is examined in the rotating diffusion cell, using an artificial lipid membrane and compared with in vitro diffusion studies using full thickness human skin as a model for the in vivo barrier to percutaneous absorption. The membrane transport of a model anionic drug, sodium salicylate and caffeine, a weakly basic drug (and hence cationic at the pHs examined) are investigated in both systems in the presence and absence of a carrier molecule, Ethomeen S12 (N,N-bis(2-hydroxyethyl) oleylamine [EtS12]).

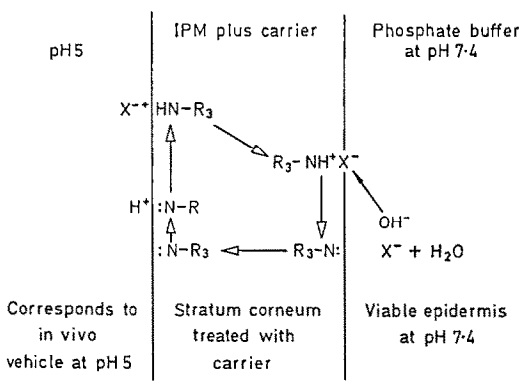

Fig. 1. The facilitated transport scheme.

Materials and Methods

Materials

Sodium salicylate (BDH), anhydrous caffeine (Sigma Chemical Co.), isopropyl myristate 98% (IPM, Sigma Chemical Co.) were used as received. Salts used for preparation of the buffers were AnalaR grade supplied by BDH (U.K.). Ethomeen S12 was a gift from Akzo Chemie U.K. Cellulose nitrate 1 μm pore size membrane filters were obtained from Whatman. Distilled water from an all glass still was used throughout.

Methods

Rotating diffusion cell studies. The rotating diffusion cell (RDC) was used to examine the transfer of caffeine and sodium salicylate across an artificial lipid membrane. This cell uses the hydrodynamics of the rotating disc to impose a known pattern of convective flow on either side of the membrane (Albery et al., 1976). The membrane consists of a cellulose nitrate membrane filter which is first rendered hydrophobic by treatment with a solution of 1,2-dimethyldichlorosilane (2%) in 1,1,1-trichloroethane. The IPM (or a solution of carrier in IPM) is then applied dropwise to the membrane. Any excess lipid is then carefully removed with a soft tissue. In previous work this has been shown to be a reproducible method of producing such membranes. In all experiments pH gradients were maintained using a pH stat technique to keep the donor compartment at pH 5, whilst the receptor compartment was maintained at pH 7.4 using Sorensen's phosphate buffer. The volumes of the donor and receptor compartments were 250 and 30 ml, respectively. The rates of appearance of salicylate and caffeine in the receptor phase were monitored continuously using a flow-through cell in a Cecil 252 UV spectrophotometer set at 298 and 274 nm, respectively. These wavelengths corresponded to the λ_{max} of the two compounds under the conditions of our experiments. Experiments were carried out at 32°C, maintained using a thermostated glass jacket surrounding the RDC. The apparent first-order rate constants were determined at least in triplicate for each rotation speed of the cell, using at least three separately prepared membranes. A lag period of at

least 1 h was allowed for equilibration before any flux measurements were determined.

Analysis of data

The flux (J) of solutes across the membrane in the RDC can be expressed as:

$$J = \vec{k} \cdot A \cdot C_d$$

where A is the effective area of the membrane, C_d is the concentration of solute in the donor compartment and $\vec{k}$ is the rate constant for transfer of the solute across the membrane, its interfaces and the stagnant diffusion layers on either side of the membrane. Determinations of J at various rotation speeds enables a plot of the inverse rate constant to be constructed as a function of the inverse square-root of the rotation speed of the cell. From this type of plot it is possible to estimate $\vec{k}$ at an infinite rotation speed where, theoretically, no stagnant diffusion layers are present on either side of the membrane.

Results

A typical graph plotted from J values obtained at the various rotation speeds of the cell is shown in Fig. 2, for caffeine transported across an IPM membrane and an IPM membrane containing 100 mM dissolved carrier. Extrapolation of the graphs to $W^{-1/2} = 0$, enables an estimation of $\vec{k}$ to be determined when no stagnant diffusion layers are present on either side of the membrane. The values of $\vec{k}$ at these conditions are reported in Table 1 for both caffeine and sodium salicylate crossing the IPM membrane in the presence and absence of carrier. From Table 1 it is seen that the carrier enhanced the transport of the salicylate anion across the membrane by a factor of approximately 3. The transport of caffeine was not enhanced by the presence of a carrier.

Further evidence for the existence of the carrier mechanism is obtained from the ability of the carrier to transport salicylate against its own concentration gradient. In a series of experiments, salicylate transport from a 200 μM pH 5 solution to a 200 μM pH 7.4 solution was monitored for a

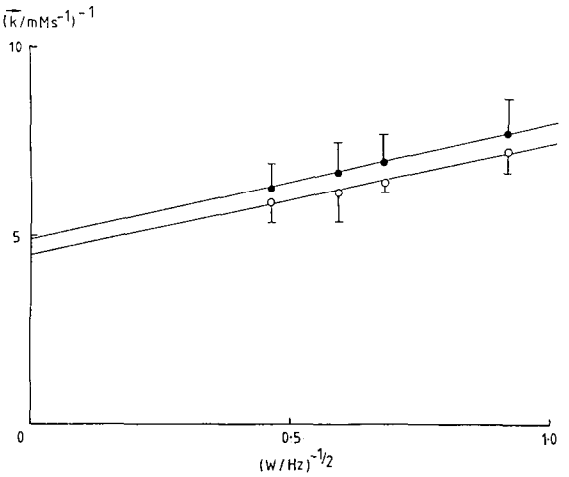

Fig. 2. Relationship between the inverse forward rate constant ($\vec{k}$) and the inverse square-root of rotation speed for caffeine traversing an IPM membrane (○) and a 100 mM EtS12 membrane (●) in the RDC at 32°C. (Half standard error bars are shown.)

period of at least 20 h at a cell rotation speed of 4.73 Hz. Under these conditions the carrier transported the salicylate anion at a rate of 0.97 nmol · $mm^{-2} \cdot h^{-1}$ ($\pm$0.09 S.E.). At the end of a typical experimental run the receptor salicylate concentration was of the order 400 μM. This phenomenon was not observed when carrier was absent from the membrane.

A facilitated transport scheme within an artificial lipid membrane has therefore been established for anionic drug molecules. It is therefore pertinent to extend this work to skin membranes in an

TABLE 1

APPARENT FORWARD RATE CONSTANTS AT $W \to \infty$ OF CAFFEINE AND THE SALICYLATE ANION TRAVERSING AN IPM MEMBRANE, IN THE PRESENCE AND ABSENCE OF 100 mM CARRIER (EtS12) WITHIN THE MEMBRANE

Solute	Membrane	$\vec{k}$ (ms^{-1})
Caffeine	IPM	2.10×10^{-7}
	100 mM EtS12	2.04×10^{-7}
Sodium salicylate	IPM	1.03×10^{-7}
	100 mM EtS12	2.86×10^{-7}

attempt to produce a coupled transport system within the stratum corneum.

In vitro skin diffusion cell studies

Full thickness human caucasian abdominal skin was obtained at autopsy, within 24 h of death, from both female and non-hairy male donors (aged 75 ± 8 years). Immediately following removal from the cadaver, the skin was separated from the subcutaneous fat by careful blunt dissection. The full thickness skin samples were either used immediately, or stored flat in sealed evacuated plastic bags at −20°C for a period not exceeding 2 weeks. When frozen skin samples were used they were allowed to thaw for a period of at least 1 h at room temperature, prior to mounting in the skin diffusion cells.

The skin cells were of conventional Franz style design and were of all-glass construction. A diffusion area of 0.5 cm^2 was available. The receptor compartment was stirred continuously by means of a teflon-coated magnetic stirring bar and contained 11 ml of degassed isotonic saline/phosphate buffer with 0.001% phenylmercuric nitrate as preservative. The receptor compartment was maintained at 37°C by immersion in a thermostated water bath. The donor compartment contained the drug which could be applied either as a thin film or as a solution. The donor compartment was maintained at ambient room temperature.

Permeation procedure

The skin samples were mounted in the diffusion cells between two ground glass flat surfaces held in position by a clamp. The skin samples were then allowed to equilibrate with the receptor solution for 1 h prior to the start of the experiments. Following equilibration the stratum corneum was impregnated with carrier applied as 50 μl of dissolved carrier (100 mM or 10 mM) in absolute ethanol. The ethanol evaporated to leave the carrier deposited on the stratum corneum. In all experiments, control cells were run where the skin samples had been treated with 50 μl of absolute ethanol. A time period of 2 h was then allowed before the drug dose was introduced to the donor compartment.

Samples of 0.5 ml were removed from the receptor compartment at appropriate time intervals and replaced with an equal volume of fresh buffer solution prethermostated to 37°C. Samples were stored at −20°C until assay. The progressive dilution of the receptor phase was taken into account in the calculations. Experiments were conducted at least in triplicate. The mean values (±S.E.) are reported for each experiment. The mean steady-state fluxes were calculated from the linear portion of the permeation versus time graphs by linear regression analysis. Permeability coefficients were calculated from the flux values obtained according to:

$$J = K_p \cdot \Delta C_s$$

where J = flux of solute per unit area, K_p = permeability coefficient and ΔC_s = concentration difference on either side of the membrane.

Analysis

Concentrations of salicylate and caffeine in the receptor phase were monitored using HPLC, consisting of a Cecil CE 2112 variable wavelength detector coupled to a Cecil CE 2010 pump. The detector was linked to a Spectra Physics computing integrator SP4100. The reverse phase column 25 cm long and 4.0 mm i.d. was packed with Partisil ODS II (10 μm particle size) and protected with a pre-column packed with Co:Pell ODS (30–38 μm particle size). The flow rate was set at 2 $ml \cdot min^{-1}$. Sample injection was made using an SGE 50 μl syringe via a Rheodyne injector.

For salicylate analysis the mobile phase consisted of acetonitrile : 0.005% orthophosphoric acid (20 : 80). The column effluent was monitored at 298 nm. Under these conditions salicylate showed a retention time of 3.2 min.

For caffeine analysis the mobile phase consisted of acetonitrile : distilled water (20 : 80). The column effluent was monitored at 274 nm. Under these conditions caffeine showed a retention time of 4.2 min.

Discussion

Salicylate permeation

Steady-state salicylate permeation was studied

by using 1 ml of 0.01 M sodium salicylate in distilled water (adjusted to pH 5 using 0.1 M HCl) in the donor compartment. In the control experiments salicylate permeation was not detectable after 96 h. The poor penetration of salicylate under these conditions is in accordance with previously reported results (Dyer et al., 1981). The barrier function of skin to salicylate was not affected by freezing or pretreatment with ethanol.

The effects on salicylate permeation following skin pretreatment with 50 μl of ethanolic solutions of EtS12 (10 mM and 100 mM) are shown in Fig. 3. Skin pretreatment with this compound markedly increased the permeability coefficient of salicylate. A lag time of about 25 h was calculated by extrapolation of the linear portions of the graphs. A reduction in the applied concentration of the enhancer produced a decreased salicylate penetration rate. The mean permeability coefficients of salicylate calculated for these experiments are given in Table 2.

The effects of the applied enhancer upon salicylate permeation following application as a thin film were also studied. The advantage of using thin film techniques in percutaneous absorption studies is that the in vivo situation is more closely mimicked than in steady-state experiments when skin hydration may alter the permeability characteristics of the skin.

TABLE 2

CALCULATED MEAN PERMEABILITY COEFFICIENTS FOR SKIN PENETRATION OF SODIUM SALICYLATE FROM A 0.01 M pH 5 SOLUTION FOLLOWING EPIDERMAL PRETREATMENT

Epidermal pretreatment	Mean permeability coefficient ($cm \cdot h^{-1}$)
Ethanol (control)	No detectable permeation
10 mM EtS12 in ethanol	4.31×10^{-4}
100 mM EtS12 in ethanol	4.29×10^{-3}

A thin film of sodium salicylate was applied to the skin surface by application of 100 μl of a 0.1 M solution at pH 5. The total dose applied was thus the same as in the previous experiment. The aqueous vehicle evaporated to leave a thin film of sodium salicylate deposited on the skin surface. The results of this experiment are illustrated in Fig. 4. In the control cells, slight salicylate permeation was achieved (a result of a more favourable concentration gradient than in the above steady-state experiment), but in those cells pretreated with EtS12, a marked increase in salicylate permeation was noted after an initial lag time of approximately 28 h. The sigmoidal shape of the curve is typical when a finite dose technique is employed, where most of the dose permeates. After 48 h 55% of the applied salicylate had permeated

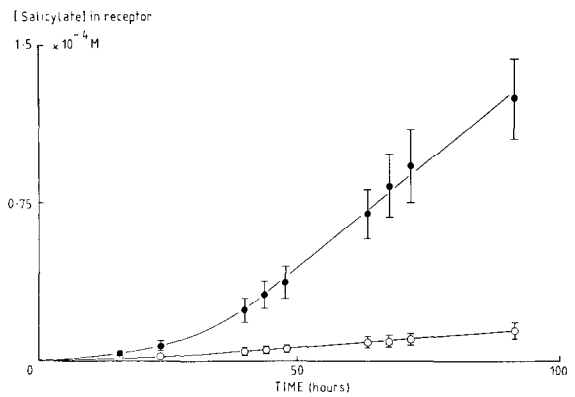

Fig. 3. Steady-state permeation profile of salicylate across full thickness human skin in vitro following skin pretreatment with 100 mM EtS12 (●) and 10 mM EtS12 (○) applied as ethanolic solutions.

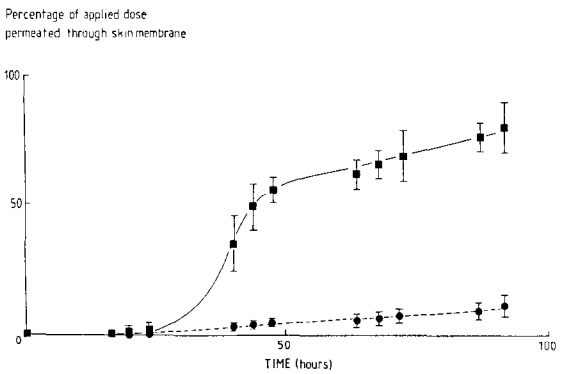

Fig. 4. Permeation of salicylate across full thickness human skin in vitro after application as a thin film. ● = control (pretreated with ethanol); ■ = skin pretreated with 100 mM EtS12 in ethanol.

in the EtS12-treated skin samples compared with only 4% permeating in the control cells. After 92 h the respective amounts permeated were 78% and 10%.

Caffeine permeation

EtS12 did not enhance the transport of caffeine across the artificial membrane. In order to determine whether EtS12 enhanced the transport of salicylate across the skin via a facilitated transport mechanism, or whether some other mechanism was responsible for this observation, the steady-state transport of caffeine across the skin was investigated under the same conditions as above.

The results of these experiments are shown following skin pretreatment with 10 mM and 100 mM EtS12 (Fig. 5). In contrast to the salicylate, caffeine readily permeated the skin in the control cells. The mean permeability coefficients are given in Table 3.

Pretreatment of the skin with EtS12 produced an enhanced permeation of caffeine, a result that was not predictable from the results obtained using the model system. EtS12 therefore exerts an effect upon the stratum corneum which may be unrelated to its carrier properties. The results serve to illustrate the care that must be exercised when using simple physicochemical models for percutaneous absorption. Simple inert lipophilic membranes are far removed from the structurally complex and perhaps less inert stratum corneum, where unpredictable interactions may take place between penetrating molecules and the cellular matrix. The development of more suitable membranes for use in such systems would be of considerable benefit. An attempt has been made to improve the membrane used in the RDC by the use of supported membranes of linoleic acid or dilute solutions of phospholipids in IPM to simulate the epidermal barrier (Guy and Fleming, 1979). These may prove to be more suitable than IPM alone for assessing the effects that compounds like EtS12 may have on percutaneous absorption.

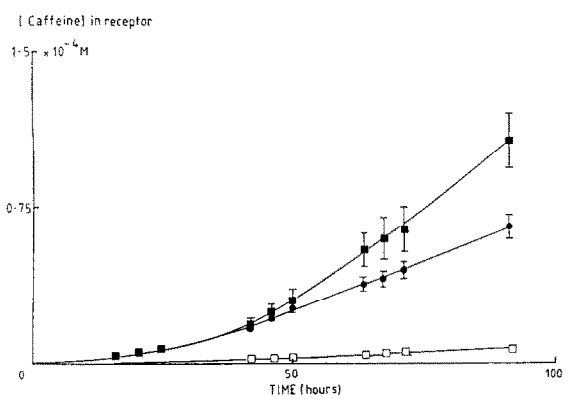

Fig. 5. Steady-state permeation profiles of caffeine across full thickness human skin in vitro. □ = control (pretreated with ethanol); ● = skin pretreated with 10 mM EtS12 in ethanol; ■ = skin pretreated with 100 mM EtS12 in ethanol.

TABLE 3

CALCULATED MEAN PERMEABILITY COEFFICIENTS FOR SKIN PENETRATION OF CAFFEINE FROM A 0.01 M pH 5 SOLUTION FOLLOWING EPIDERMAL PRETREATMENT

Epidermal pretreatment	Mean permeability coefficients ($cm \cdot h^{-1}$)
Ethanol (control)	1.14×10^{-4}
10 mM EtS12 in ethanol	2.48×10^{-3}
100 mM EtS12 in ethanol	4.89×10^{-3}

A potentially useful class of penetration enhancer has been identified, that of the ethoxylated long-chain amines, although no toxicity studies have been attempted. Related compounds to EtS12 are now being investigated for accelerant properties in the percutaneous absorption process. Further work is expected to establish their suitability for use as penetration enhancers in man.

Acknowledgement

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