J. Pharm. Pharmacol. 1995, 47: 26–29
Received May 13, 1994
Accepted July 7, 1994

Measuring the Diffusional Pathlength and Area Within Membranes of Excised Human Stratum Corneum

RENATE LIECKFELDT AND GEOFFREY LEE

Department of Pharmaceutical Technology, University of Erlangen, Erlangen, Germany

Abstract

By using lipid-free membranes it was possible to measure the extended diffusional pathlength (L*) and reduced diffusional area (A*) within samples of human stratum corneum.

The value of 883 obtained for the factor L*A/LA* (A = external membrane area and L = membrane thickness) compared well with previously published theoretical values. It was also consistent with measurements of lag-time and permeability made on both lipid-free and intact stratum corneum.

The geometrical barrier formed by the corneocytes was found to be quantitatively of equal importance to the extra-cellular lipid barrier for maintaining the overall barrier function of the stratum corneum.

The barrier property of human stratum corneum is a consequence of its internal structure. The presence of numerous layers of thin, very broad corneocytes presents a primary geometrical barrier to permeating molecules. The lipid phase of continuous bilayers forms a substantial diffusional barrier within the extracellular channels. Although much is made in the literature of the crucial importance of the composition and microstructure of the lipid, the geometrical barrier, although recognized, is treated as playing but a secondary role. Yet its vital part in maintaining the stratum corneum barrier has been known for some time. Michaels et al (1975) showed how a 'brick-and-mortar' arrangement of the corneocytes is responsible for reducing permeation rates compared with a homogeneous membrane. Albery & Hadgraft (1979) recognized that the tortuous intercorneocyte diffusional pathlength, L*, must be greater than the stratum corneum thickness, L, and that the available diffusional area, A*, must be smaller than its external surface, A. The effect of these two geometrical factors is to hinder the passage of a drug through the membrane, provided the corneocytes are less permeable than the lipid phase. Calculations using steady-state models for a heterogeneous membrane show that their combined influence, expressed as L*A/LA*, should reduce the flux by a factor of 1000 (Albery & Hadgraft 1979; Cussler et al 1988). Separating the two factors, pathlength and area, yields values of up to approximately 30 for L*/L and 1/100 for A*/A (Lange-Lieckfeldt & Lee 1992), depending on corneocyte dimensions.

Confirmation of these theoretical values by direct experimental measurement using stratum corneum membranes has, however, proved elusive. Potts & Francoeur (1991) measured steady-state water permeation through intact stratum corneum of pig and determined a value of approximately 50 for L*A/LA*, although this was attributed to increased pathlength alone. The uncertainty with the method used is inherent in Fick's first law; the partition coefficient (K) between the extracellular lipid channels of the stratum corneum and buffer solution must be known before L*A/LA* can be calculated from the steady-state flux. Potts & Francoeur (1991) indirectly estimated K from thermal analysis data. We have used a simple technique to avoid this problem, which gives, we feel, a reliable and direct estimate of L*A/LA* within human stratum corneum. The permeation rate of a model drug through lipid-free stratum corneum membranes is determined, which avoids use of an estimated partition coefficient. Because permeation is rapid, the result must first be evaluated with a non-sink model, before L*A/LA* can be calculated from Fick's first law. The method is not without technical difficulties, because of the mechanical frailty of the lipid-free stratum corneum. Nevertheless, the value for L*A/LA* is found to be in good agreement with theory. This factor can be used to illustrate quantitatively the relative importance of the lipid and geometrical barriers within stratum corneum membranes.

Correspondence: G. Lee, Lehrstuhl für Pharmazeutische Technologie, Cauerstr. 4, 91058 Erlangen, Germany.

Materials and Methods

Measurement of permeation rate through stratum corneum membranes

Whole human skin was excised from the upper thigh and treated at 60°C for 2 min in water (Kligman & Christophers 1963), followed by digestion of the viable epidermis with trypsin to yield stratum corneum. The permeation rate of the basic model drug tiamenidine (pK_a = 8·3; Hoechst AG, Frankfurt, Germany) through both intact and lipid-free stratum corneum membranes was then determined using a horizontal-type, infinite-dose diffusion cell, as previously described (Göpferich & Lee 1991). Buffer (pH 5) was used for both donor and acceptor phases, and the tiamenidine content of the latter determined by HPLC. Each result was expressed as a permeation profile of drug mass in the acceptor phase, $m_a(t)$, vs time, t. Lipid-free stratum corneum membranes were prepared from intact stratum corneum by treatment with a chloroform/methanol mixture (20 : 80), which is known to remove both the polar and non-polar lipids (Sweeney & Downing 1970). The resulting

lipid-free membrane was, however, very delicate and folded readily, rendering handling extremely difficult. In this condition it was not possible to attach the membrane within the diffusion cell without its tearing. To overcome this problem, an intact membrane was placed within the cell before extraction of its lipids, and both donor and acceptor compartments filled with solvent. After 30 min, the solvent was removed without disassembly of the diffusion cell, which was then rinsed numerous times first with acetone and then pH 5 buffer, until the eluate was found by HPLC to be lipid-free. The donor and acceptor compartments were then filled as before.

Measurement of drug diffusivity in buffer

For the calculation of L*A/LA* it was necessary to know the diffusivity of tiamenidine within the pH 5 phosphate buffer. This was determined using a standard diaphragm cell method (Mills & Woolf 1968), with 1·0 M HCl (ionic diffusivity = $3{\cdot}436 \times 10^{-5}$ cm^2 s^{-1} at 25°C) serving to calibrate the sintered glass membrane.

*Calculation of L*A/LA* for excised human stratum corneum*

L*A/LA* is related to the steady-state flux, $\Delta m(t)/\Delta t$, by Fick's first law:

$$\Delta m(t)/\Delta t = [KD_{lip}c_o a/h]\cdot[L^*A/LA^*] \quad (1)$$

where K = stratum corneum lipid channel/buffer partition coefficient, D_{lip} = diffusivity within extracellular lipid pathway, c_o = concentration in donor solution, a = membrane area and h = membrane thickness. The difficulty of obtaining an accurate value for K was overcome by using a lipid-free stratum corneum membrane. Permeation is now assumed to occur through the extracellular channels, which are filled with buffer. Fick's first law is still valid in the steady-state:

$$\frac{\Delta m(t)}{\Delta t} = \frac{D_w c_o K A^*}{L^*} \quad (2)$$

where D_w is diffusivity within the buffer, and K is now the partition coefficient between the buffer within the extracellular channels and the buffer of the donor solution, i.e. K = 1 (see Fig. 1). L* and A* are, of course, unknown. If, in their place, we substitute the directly accessible values for L and A, the effective diffusivity within the membrane, D_{eff}, must be taken (Lange-Lieckfeldt & Lee 1992), giving:

$$\frac{\Delta m(t)}{\Delta t} = \frac{D_{eff} c_o K A}{L} \quad (3)$$

As the permeation rate is the same in each case, we obtain (Lange-Lieckfeldt & Lee 1992):

$$\frac{L^*}{L}\frac{A}{A^*} = \frac{D_w}{D_{eff}} \quad (4)$$

D_w was available from the diaphragm cell. D_{eff} for the lipid-free stratum corneum membranes had to be calculated by fitting the measured permeation profile of $m_a(t)$ vs t (using A and L) to the applicable solution of the linear diffusion equation for membrane-permeation under non-sink conditions, as described previously (Göpferich & Lee 1991). The non-sink conditions were established very rapidly in the acceptor compartment as a result of the high permeation rate. L was measured for each stratum corneum membrane at 20 places using an Elcometer (Elcometer, Manchester, UK). A was determined from the available diffusional area of each diffusion cell. Substitution of these values into equation 4 yielded L*A/LA*.

Results and Discussion

Measurements of transepidermal water loss are frequently cited as proof of the overriding importance of the lipid structure for the stratum corneum barrier. Solvent-extraction of the stratum corneum lipids causes, for example, an approximate 10-fold increase in the rate of transepidermal water loss (Elias 1991). This attenuation of the stratum corneum barrier is reflected in the present study by the two typical permeation profiles shown in Fig. 2 for intact and lipid-free stratum corneum membranes. Removal of the lipids causes the lag-time, τ, to fall by at least two orders of magnitude to below 0·1 h. Lag-times of this order are too small to be accurately determined from the permeation profile, giving the misleading impression that the stratum corneum barrier has been essentially destroyed. This impression is confirmed by the sharp increase in the steady-state permeability, P, by some three orders of magnitude up to approximately 0·2 cm h^{-1}.

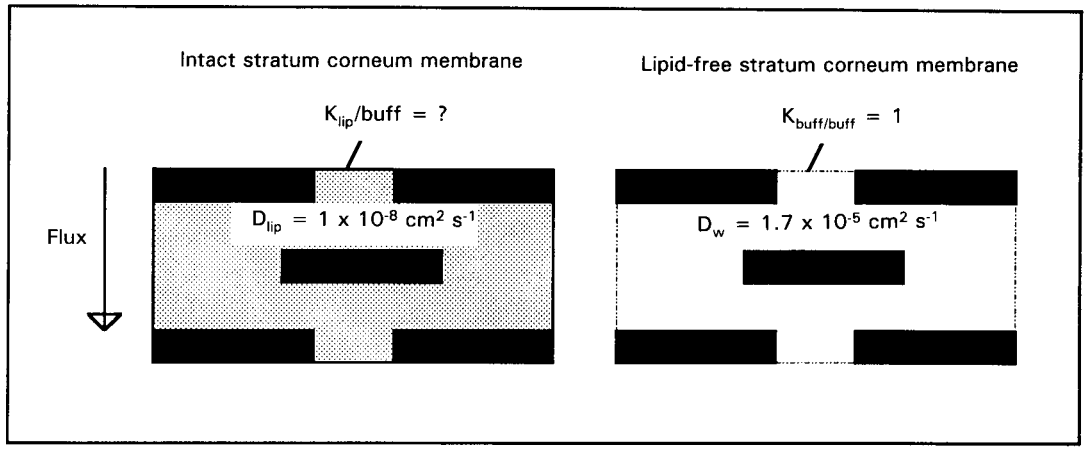

FIG. 1. Illustration of factors influencing permeation through intact and lipid-free membranes.

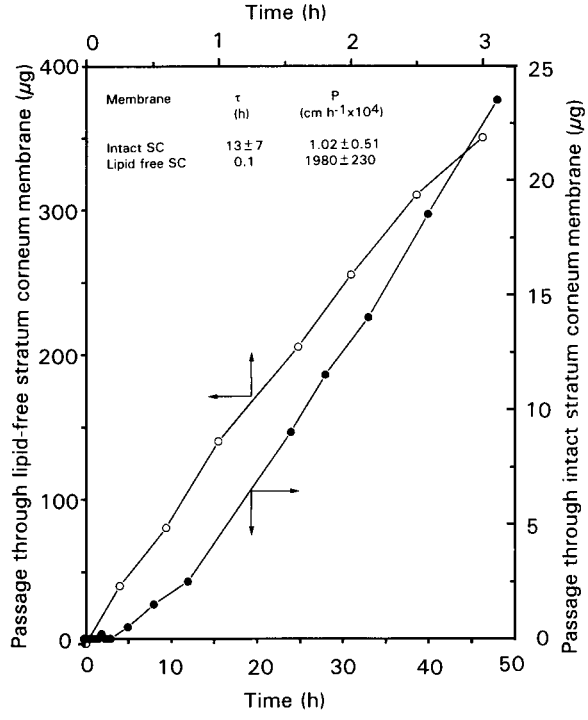

FIG. 2. Permeation profiles of $m_a(t)$ vs t for passage of tiamenidine through intact (●) and lipid-free (○) stratum corneum membranes. Average thickness, L, of intact membranes was 8 μm and of lipid-free membranes 3·7 μm. In all cases n = 4.

Table 1. Calculated values for L*A/LA* for three replicate permeation measurements on lipid-free human stratum corneum. D_w was determined in pH 5 buffer using diaphragm cell = $1·7 \times 10^{-5}$ cm^2 s^{-1}.

Sample	L (cm × 10^{-4})	τ (h)	D_{eff} (cm^2 s^{-1} × 10^{-8})	D_w/D_{eff} = L*A/LA*
1	3·5	< 0·1	1·99	940
2	3·7	< 0·1	2·13	878
3	3·8	< 0·1	2·25	831
Mean				883 ± 55

Yet, the mean value of 883 obtained for L*A/LA* using the lipid-free membranes (Table 1) proves that the geometrical barrier of the corneocytes is still present, i.e. the stratum corneum barrier was not fully attenuated by removing the lipids. This value is exactly of the order of magnitude predicted by steady-state models for flake (Cussler et al 1988) and brick-and-mortar geometries (Michaels et al 1975; Lange-Lieckfeldt & Lee 1992) under the premise of impermeable corneocytes. As the exact internal dimensions of the stratum corneum membranes used here—number of corneocyte layers, corneocyte width and height, lipid content—are unknown, it is not possible to calculate their exact, theoretical L*A/LA*. Nevertheless, interpretation of this result is clear: the combined effects of increased diffusional pathlength and reduced diffusional area within the stratum corneum give rise to a geometrical barrier of dimensionless magnitude approximately 10^3 in the total barrier property of the stratum corneum. The influence of this geometrical barrier on P and τ will be different. According to Fick's first law, P is directly proportional to L*A/LA*. The geometrical barrier will reduce P, therefore, by approximately three orders of magnitude compared with a homogeneous membrane. As already seen in Fig. 2, this effect is of the same magnitude as the influence of the lipid barrier. The effect of the geometrical barrier on τ depends only on increased pathlength, τ being independent of the diffusional area. Although L*/L cannot be separated out of the value obtained for L*A/LA*, we can refer to calculations with a steady-state model (Lange-Lieckfeldt & Lee 1992), which gave values between 10 and 30 for stratum corneum membranes containing 10 corneocyte layers. For L*/L = 20, for example, the result would be an increase in τ by two to three orders of magnitude according to:

$$\tau = L^2/6D \qquad (5)$$

Again, this is of the same magnitude as the influence of the lipid barrier (Fig. 2). It is thus evident that the geometrical barrier, when viewed quantitatively, is of equal importance to the lipid barrier for maintaining the overall barrier property of the stratum corneum.

Table 2. Model calculations to illustrate the origin of the stratum corneum barrier, presuming impermeable corneocytes. D_w = drug diffusivity in water; D_{lip} = drug diffusivity in lamellar gel phase; L* = diffusional pathlength within stratum corneum of thickness L; A* = diffusional area within stratum corneum of area A; c_o = drug concentration in donor solution; $\Delta m/\Delta t$ = flux through membrane. Membrane C represents intact stratum corneum having both lipid and geometrical barriers. Membrane B represents lipid-free stratum corneum, where only the geometrical barrier exists. Membrane A has no barrier property and represents free diffusion in water.

Model	Membrane C intact stratum corneum	Membrane B lipid-free stratum corneum	Membrane A no barrier
L (μm)	15	15	15
A (cm^2)	1	1	1
c_o (mg L^{-1})	1	1	1
D (cm^{2}s^{-1})	$D_{lip} = 1 \times 10^{-8}$	$D_w = 1·7 \times 10^{-5}$	$D_w = 1·7 \times 10^{-5}$
L*A/LA*	890	890	1
L*/L	20†	20†	= A/A* = 1
Lag time	$((L^*/L)L)^2/6D_{lip}$ = 4·2 h	$((L^*/L)L^2)/6D_w$ = 9 s	$L^2/6D_w$ = 0·022 s
Permeability (cm h^{-1}) = $\Delta m/\Delta t \cdot a \cdot c_o$	$D_{lip}/(L^*A/LA^*)L = 7·3 \times 10^{-5}$	$D_w/(L^*A/LA^*)L = 4·6 \times 10^{-2}$	$D_w/L = 41·8$

† Lange-Lieckfeldt & Lee (1992).

The value of $L^*A/LA^* = 883$ obtained here is subject to some qualification. It is not known, for example, if the uptake of buffer by the stratum corneum also leads to swelling of the corneocytes and consequently channel narrowing. This could falsify the value for L^*A/LA^*, by making both L^* and A^* smaller than they are within intact stratum corneum. The assumption of impermeable corneocytes is also only an approximation. Calculations using a non-steady-state model for stratum corneum (Lieckfeldt et al 1993) suggest, however, that the relative permeability of the corneocytes/lipid phase is $< 10^{-4}$. Because of these uncertainties, we must check if the values obtained here for L^*A/LA^*, P and τ are consistent with membrane-diffusion theory. We consider the three membrane structures summarized in Table 2. The homogeneous, lipid-free membrane A clearly has no barrier property. Diffusion occurs through water ($D_w = 1{\cdot}7 \times 10^{-5}\,cm^2\,s^{-1}$, from Table 1) and there is no geometrical barrier ($L^*A/LA^* = 1$). τ is calculated to be some 22 ms and P $42\,cm\,h^{-1}$. We now introduce into this membrane first a geometrical barrier and then a lipid barrier. For membrane B, we take $L^*A/LA^* = 883$ (from Table 1) and $L^*/L = 20$ (from Lange-Lieckfeldt & Lee 1992). D_w remains the same as with membrane A, since the membrane is still lipid-free. τ increases to 9 s and P falls to $0{\cdot}05\,cm\,h^{-1}$. Both of these values are in reasonable agreement with the results for the lipid-free stratum corneum membrane in Fig. 2. With membrance C, we replace the extracellular aqueous phase with lipid phase, to represent now both geometrical and lipid barriers, i.e. intact stratum corneum. We take a diffusivity of $D_{lip} = 10^{-8}\,cm^2\,s^{-1}$ determined for a lamellar gel phase mixture of cholesterol, ceramide and fatty acid (Lieckfeldt et al 1993). τ now increases to 4·2 h and P is reduced to $< 10^{-4}\,cm\,h^{-1}$, both of which, again, correspond reasonably well to our measured values for intact stratum corneum in Fig. 2, bearing in mind that $K_{sc/buffer}$ is ignored. Although these calculations are very simple order-of-magnitude approximations, the result obtained for L^*A/LA^* is shown to be consistent with a simple two-phase model for stratum corneum. The results illustrate simply and clearly the origins of the barrier property of the stratum corneum at the macro-molecular level.

References

Albery, W. J., Hadgraft, J. (1979) Percutaneous absorption: theoretical description. J. Pharm. Pharmacol. 31: 129–139

Cussler, E., Hughies, S., Ward, W., Aris, R. (1988) Barrier membranes. J. Membr. Sci. 38: 161–174

Elias, P. (1991) Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. J. Contr. Rel. 15: 199–208

Göpferich, A., Lee, G. (1991) Measurement of drug diffusivity in stratum corneum membranes and a polyacrylate matrix. Int. J. Pharm. 71: 245–253

Kligman, A., Christophers, E. (1963) Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88: 702–710

Lange-Lieckfeldt, R., Lee, G. (1992) Use of a model lipid matrix to demonstrate the dependence of the stratum corneum's barrier properties on its internal geometry. J. Contr. Rel. 20: 183–194

Lieckfeldt, R., Villalaín, J., Gómez-Fernández, J.-C., Lee, G. (1993) Diffusivity and structural polymorphism in some model stratum corneum lipid system. Biochim. Biophys. Acta 1151: 182–188

Michaels, A., Chandrasekaran, S., Shaw, J. (1975) Drug permeation through human skin: theory and in vitro experimental measurement. AIChE J. 21: 985–995

Mills, R., Woolf, L. (1968) The Diaphragm Cell. Australian National University Press, Canberra

Potts, R., Francoeur, M. (1991) Influence of stratum corneum morphology on water permeability. J. Invest. Dermatol. 96: 495–499

Sweeney, T., Downing, D. (1970) The role of lipids in the epidermal barrier to diffusion. J. Invest. Dermatol. 55: 135–140