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Factors influencing hydrocortisone permeation into human hair follicles: Use of the skin sandwich system

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ABSTRACT

The aim of the present study was to use the in vitro human skin sandwich system in order to quantify the influence of formulation variables on intrafollicular hydrocortisone permeation. The investigated variables were the pH and the viscosity of the topical formulation as well as the presence of chemical enhancers (carvone, menthone, oleic acid and sodium lauryl sulphate). Furthermore, skin sandwich hydration was also varied in order to determine if the method itself can be run using only partially hydrated skin tissues. It was determined that the follicular contribution to hydrocortisone flux decreased marginally with increasing alkalinity in the pH range 3–8.8. Intrafollicular penetration was markedly reduced when HPMC gels were used instead of an aqueous solution. Pretreating the skin with chemical enhancers also reduced the follicular contribution to flux, probably due to permeabilisation of the continuous stratum corneum. Furthermore, it was not possible to satisfactorily modify the skin sandwich method so that it could be deployed using less hydrated skin.

1. Introduction

Historically, researchers studying the cutaneous delivery of drugs have debated the relative contribution of penetration through the continuous stratum corneum as opposed to penetration through the hair follicles. Early measurements of follicular orifices indicated that these accounted for only ~0.1% of the total skin surface area. Thus, for many years, it was believed that follicular transport represented a negligibly small fraction of total drug absorption (Scheuplein, 1967; Scheuplein et al., 1969). Yet, more contemporary studies have indicated that the hair follicles might mediate a greater role than previously believed (Meidan et al., 1998; Ogiso et al., 2002; Dokka et al., 2005; Jung et al., 2006; Teichmann et al., 2006; Otberg et al., 2007; Lademann et al., 2007). This paradigm shift has been triggered by the realisation that the hair follicle actually represents an epidermal invagination that reaches the dermis. Hence, there is really a greater actual surface for potential absorption (Meidan et al., 2005).

Until recently, research in this area was hampered by the fact that there were no suitable techniques that allowed quantification of follicular drug transport. Various types of animal skin studies have been conducted but interpreting the results of these is always problematic. Specifically, it is difficult to ensure that the barrier properties of any follicle-containing membrane are identical to those of the "control" follicle-free membrane. Advanced imaging systems, which are continually improving, have provided important information (Grams et al., 2005; Stracke et al., 2006; Jacobi et al., 2007). However, these tend to yield a static view of what is essentially a highly dynamic process. Very recently, Lademann and colleagues have developed various novel follicular orifice-blocking (Jung et al., 2006; Teichmann et al., 2006; Otberg et al., 2007; Lademann et al., 2007) as well as differential stripping (Ossadnik et al., 2007) approaches. Furthermore, Abdulmajed and Heard (2007) applied an interesting follicle-sealing technique in recent experiments. All these methodologies have generated an appreciable interest although some may require specialist expertise to implement.

One recently devised quantitative technique is the in vitro skin sandwich method (Barry, 2002; Essa et al., 2002). Basically, the role of the 'shunts' in total percutaneous absorption is elucidated by comparing drug flux across hydrated epidermal membrane with that through a hydrated 'sandwich' of epidermal membrane plus adhering extra stratum corneum on top. As Fig. 1 illustrates, the overlying stratum corneum blocks all available shunts such as hair follicles. If 'shunts' do not contribute to permeation then steady-state flux through the 'sandwich' is half that of the single membrane. Conversely, if shunts facilitate all drug transport then flux through the 'sandwich' is zero. Thus, the extent of flux decrease facilitates quantification of the shunt contribution to total absorption. Crucially, the shunts represent hair follicles since the much

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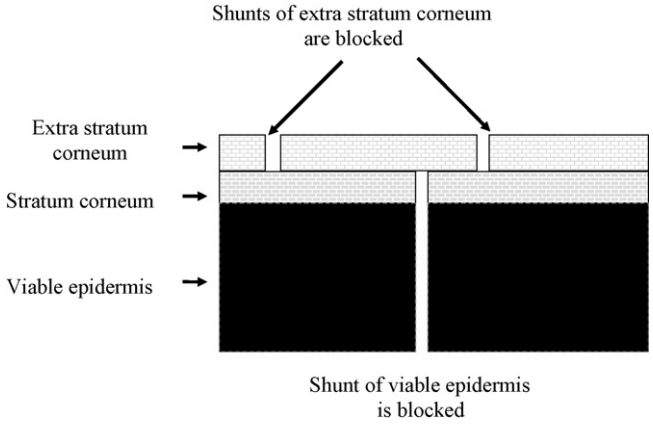

Fig. 1. Scheme illustrating the basis of the skin sandwich method.

smaller sweat duct openings shut in highly hydrated skin. Complete algebraic descriptions, analysis and validations of the skin sandwich system have been published (Barry, 2002; Essa et al., 2002).

In a previous study, the human skin sandwich system was used to screen several solutes in order to quantify the role of partition coefficient on follicular drug absorption (Frum et al., 2007). It was found that when applied as a saturated aqueous solution, an appreciable 46% of the hydrocortisone that permeated, entered the skin through follicular orifices. Although aldosterone and deoxyadenosine also demonstrated a considerable follicular component, we decided to study hydrocortisone as it is has been more extensively studied within the percutaneous delivery context. Thus, the aim of the present study is to examine the influence of topical vehicle formulation variables on the follicular component of hydrocortisone penetration. The investigated donor vehicle parameters are pH, viscosity and the presence of chemical enhancers – carvone, menthone, oleic acid and sodium lauryl sulphate. We also test skin sandwich hydration in order to see if the system itself can be run using only partially hydrated skin samples.

2. Materials and methods

2.1. Chemicals

Hydrocortisone, (+)-carvone, menthone, oleic acid, hydroxypropyl methylcellulose (HPMC: viscosity of 4000 cps for 2% aqueous solution at 20 °C), propylene glycol, sodium hydrogen carbonate, bovine pancreatic trypsin (T-4665) and phosphate buffer saline (PBS) tablets (pH 7.4) were all purchased from Sigma-Aldrich (Poole, UK). Tritiated [1,2,6,7-^{3}H]-hydrocortisone (74 Ci/mmol) was obtained from Amersham Biosciences (Little Chalfont, UK). Sodium lauryl sulphate (SLS) was purchased from J.M. Loveridge Ltd. (Southampton, UK). Sodium azide was supplied by Acros Organics (Geel, Belgium). Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, phosphoric acid and sodium chloride were all obtained from VWR (Lutterworth, UK). Potassium chloride and scintillation fluid (Optiphase HiSafe 3) were supplied by Fisher Scientific (Loughborough, UK). Scintillation vials were purchased from Fisher Packard Instrument Co. (Meriden, CT). Double distilled, deionised water was employed throughout.

2.2. Preparation of topical formulations

This study involved the preparation and characterization of three distinct formulation types. These were; hydrocortisone in PBS at pH 7.4, hydrocortisone in different buffer saline systems (pH 3, 6 and 8.8) and hydrocortisone-containing HPMC gels (0.6% and 1%, w/v). These were, respectively, employed, as a 'standard' reference formulation, to assess the influence of pH and to assess the influence of viscosity.

2.2.1. Saturated hydrocortisone solution in PBS

In order to allow for maximal thermodynamic activity, hydrocortisone was prepared as a saturated solution in PBS. This was achieved by adding excess of the 'cold' hydrocortisone to PBS and stirring for 48 h. The solution was subsequently filtered through a 6 μm pore cellulose membrane filter (Whatman Ltd., Brentford, UK). Finally, the 'cold' hydrocortisone solution was spiked with a small volume of tritiated hydrocortisone so that each prepared donor solution exhibited an activity of 5 μCi/ml. Although the addition of the small volume was technically diluting the solution, the deviation from saturation was negligibly small in practice.

The value of hydrocortisone concentration at saturation was determined by applying a radioactivity-based methodology. Approximately 100 mg 'cold' hydrocortisone was dissolved in 20 ml of ethanol and spiked with an aliquot of tritiated hydrocortisone (20 μCi). This was thoroughly mixed to provide a radiolabelled hydrocortisone solution. Liquid scintillation counting of serial dilutions of this solution yielded a standard curve relating disintegrations per minute to total drug mass. To determine saturation solubility in PBS, the remaining solution of hydrocortisone was evaporated to dryness overnight and 10 ml of PBS (pH 7.4) was added. The resultant solution was stirred for 48 h at 32 °C and filtered through a cellulose membrane filter (Whaman Ltd, Brentford, UK) of pore size 6 μm. This solution was then assayed by liquid scintillation counting. This determination of hydrocortisone solubility in PBS was made in triplicate.

2.2.2. Saturated hydrocortisone solutions in different buffer systems

To prepare phosphate buffer saline systems with different pH values but similar in composition to PBS tablets (pH 7.4), it was necessary to formulate buffer systems (KH_2PO_4/K_2HPO_4 and H_3PO_4/KH_2PO_4) with a 0.01 M strength. Each constituent of the buffer system was titrated against the other to yield buffer systems with the following pH values: 3.0, 6.0, 7.4 and 8.8. Each buffer system also contained 0.0027 M potassium chloride and 0.137 M sodium chloride. Titration procedures gave an indication of buffer capacity at each target pH value. The KH_2PO_4/K_2HPO_4 buffer system was relatively stable at pH 6.0 and 7.4 while the H_3PO_4/KH_2PO_4 buffer system was relatively stable at pH 3.0. However, the plots indicated that the KH_2PO_4/K_2HPO_4 buffer system was potentially unstable at pH 8.8. Hence, for these transport experiments, donor solution pH was measured both before and after each individual transport study. These measurements validated that the donor solutions were maintained at pH8.8.

Hydrocortisone was prepared as a saturated solution in each buffer solution in the same manner as described above (Section 2.2.1). Since altering the pH of solutions containing neutral solutes does not significantly alter solubility values (Hughes et al., 2005), this value was also applicable for all the buffers.

2.2.3. Use of HPMC gels

Initially, a saturated solution of 'cold' hydrocortisone in PBS (pH 7.4) was prepared in the manner described above. This solution was then spiked with an aliquot of tritiated hydrocortisone solution in order to obtain a solution exhibiting an activity of 10 μCi/ml. This radiolabelled solution was then gradually added to a weighed amount of HPMC powder over a period of approximately 1 h. During this time, the blend was thoroughly mixed with a Model SL2T homogenizer (Silverson machines, Chesham, UK) until a homogenous gel was formed. Two different HPMC gels were prepared and

these exhibited a final HPMC concentration of 0.6 and 1% (w/w), respectively. Each gel was stored at room temperature for at least 24 h prior to rheological measurements.

Rheological (continuous shear) measurements were performed on each gel using a CSL 100 Rheometer (TA Instruments, Crawley, UK). Flow curves were obtained at 32 ± 0.1 °C corresponding to the temperature of the skin surface in the Franz cell system. A cone and plate of 4 cm diameter and 2° angle were employed. All measurements were determined in automatic mode with each sample exposed to increasing and decreasing shear rate sweep (0.3–10 s^{-1}) with a 6 min sweep time (3 min up; 3 min down). Sample evaporation and draughts were minimized by using an anti-evaporation unit, consisting of a vapor hood and solvent trough. Apparent viscosity was calculated at the apex of each flow curve. Experiments were conducted in triplicate.

A Polyvar (Reichert–Jung, Austria) microscope was used in conjunction with a Linkam TMS 91 hot stage (Tadworth, UK) to view each gel formulation at 32 °C. Each sample was observed under crossed polarisers.

2.3. Preparation of chemical enhancer solutions

Propylene glycol was chosen as a solvent for all the tested enhancers because of its common use as a solvent in dermatological preparations (Gao and Singh, 1998; Vaddi et al., 2002; Kang et al., 2007). Sodium lauryl sulphate was formulated as a 1% (v/v) solution in propylene glycol. This concentration was selected since it is an FDA-approved concentration that is widely deployed in many topical cosmeceutical products (Porter, 1994). All the other chemical enhancers were formulated as 5% (v/v) solutions.

2.4. Preparation of skin membranes

Full-thickness cadaveric human skin samples were obtained from the National Disease Research Interchange (Philadelphia, PA). The skins were derived from the abdominal region of five Caucasian females of mean age 69 years and an age standard deviation of 14 years. The supplied skin sections were stored at −80 °C for a period of up to 6 months. Prior to each permeation study, the skins were thawed at room temperature and epidermal membranes were prepared according to the established heat separation method (Kligman and Christophers, 1963). Essentially, excess fat and adipose tissue were removed and skin sections were immersed for 45 s in a water bath maintained at 60 °C. The epidermis was then gently teased off the underlying dermis and floated on 0.002% (w/v) sodium azide solution. Stratum corneum (SC) membranes were prepared according to the methodology described previously (Frum et al., 2007). Epidermal membranes were floated with the epidermal side down on an aqueous solution containing 0.0001% trypsin and 0.5% (w/v) sodium hydrogen carbonate maintained at 37 °C. After 12 h, the membranes were picked up on filter paper and remaining digested cells were washed off with water. The SC membranes were then floated on water for 2 h to remove residual digested cells and trypsin.

In the case of chemical enhancer pretreatment studies, an extra methodological step was implemented. Epidermal and SC membranes of approximately 2 cm^2 surface area were floated SC side down on 10 ml of test chemical enhancer-propylene glycol solution. After about 1 h, the membranes were picked up on filter paper and washed with water.

2.5. Hydration of the membranes

Hydration of the membranes was a two-part process. Initially, epidermal and SC membranes were floated with the SC side uppermost on 0.002% sodium azide solution for at least 24 h. Thereafter, SC membranes were placed upon epidermal membranes derived from adjacent skin regions in order to produce SC/epidermal sandwiches.

The second part of the hydration process involved the use of static Franz diffusion cells (PermeGear, Bethlehem, PA), exhibiting a diffusional area of 0.64 cm^2 and a receptor compartment volume of 5.3 ml. An aqueous solution containing 0.002% (w/v) sodium azide was degassed by ultrasonication (Camlab Transsonic T310, Cambridge, UK) for 15 min. The receiver compartments were filled with this solution while epidermal or sandwich membranes were inserted as barriers with the stratum corneum side uppermost. The receiver phase was stirred at 600 rpm and maintained at 37 ± 0.5 °C by a thermostatically regulated water pump (Haake DC10, Karlsruhe, Germany) that circulated water through each chamber jacket. The donor compartments were filled with the same preservative solution and subsequently sealed with Parafilm. The Franz cells were left for 24 h to facilitate virtually full hydration of the membranes. Subsequently, the preservative solution in the donor compartment was removed so that transport studies could be initiated.

In order to examine the influence of skin sandwich hydration, experiments involving the use of partially hydrated skin membranes were also undertaken. For these experiments, there was no membrane floatation on dilute sodium azide solution. Instead, the prepared membranes were inserted as barrier membranes on the Franz cells with the skin surface left open to the atmosphere for 12 h.

2.6. Permeation studies

The protocol involved adding 200 μL of saturated hydrocortisone solution or 200 mg of hydrocortisone-containing HPMC gel on to either epidermal or sandwich membranes mounted in Franz cells. At selected time points, a 100 μl aliquot of solution was withdrawn from each receiver cell and replaced with the same volume of blank receiver solution. Each 100 μL aliquot was vortexed with 3 ml of scintillation fluid and assayed for tritiated test penetrant content using a liquid scintillation counter (Packard, TriCarb™ 1600TR). The machine had been pre-calibrated for tritium measurement by the use of internal standards while the assay had been validated by running calibration curves of DPM versus tritiated hydrocortisone concentration. Measured activity values were converted into concentration values and these were then corrected for progressive dilution in the regular manner (Khan et al., 2005). Permeation was allowed to proceed for 28 or 52 depending upon the duration required to achieve steady-state flux.

Percentage follicular contribution was determined according to the equation:

$$\%\,\mathrm{FC} = \left[1 - \left(\frac{2 \times J_{\mathrm{Sand}}}{J_{\mathrm{Ep}}}\right)\right] \times 100 \quad (1)$$

where % FC is the percentage follicular contribution to total percutaneous absorption, J_{Sand} and J_{Ep} are the steady-state flux values for the sandwich and single epidermis, respectively. Each permeation study consisted of three to five experimental replicates.

Data was evaluated using the two-sided Student's t test with statistical significance set at $P < 0.05$. All statistical analysis was performed using the IBM-compatible software package Minitab™ (Minitab Inc., State College, PA).

3. Results and discussion

Before interpreting the results, it is important to understand the exact nature of the information that is derived from the skin sand-

Table 1
The Influence of topical vehicle composition on hydrocortisone permeation parameters

Hydrocortisone-containing donor formulation	J_{Ep} (ng cm^{-2} h^{-1})	Epidermal k_p (cm h^{-1} × 10^{-4})	J_{Sand} (ng cm^{-2} h^{-1})	J_{Sand}/J_{Ep}	Follicular contribution (%)
PBS at pH 3.0	29.54 ± 3.27	0.94	6.87 ± 0.19	0.23	54
PBS at pH 6.0	49.52 ± 13.81	1.68	11.81 ± 0.86	0.24	52
PBS at pH 7.4	34.09 ± 8.91	1.16	9.20 ± 2.83	0.27	46
PBS at pH 8.8	112.64 ± 5.85	3.82	31.47 ± 12.43	0.28	44
HPMC:PBS at pH 7.4 (0.6:99.4)	6.54 ± 2.63	0.22	3.02 ± 1.42	0.46	8
HPMC:PBS at pH 7.4 (1.0:99.0)	9.74 ± 3.49	0.33	4.08 ± 1.60	0.42	16
PBS at pH 7.4 + oleic acid pretreatment	543.79 ± 113.09	18.46	184.05 ± 44.82	0.34	32
PBS at pH 7.4 + menthone pretreatment	38.97 ± 8.73	1.32	14.85 ± 3.43	0.38	24
PBS at pH 7.4 + carvone pretreatment	27.42 ± 3.19	0.93	15.18 ± 6.73	0.55	−10[a]
PBS at pH 7.4 + SLS pretreatment	43.28 ± 3.32	1.47	24.32 ± 0.99	0.56	−12[a]
PBS at pH 7.4 with partially hydrated skin	50.69 ± 4.09	1.72	9.82 ± 3.23	0.19	62

[a] See text for explanation.

wich method. This technique quantifies how much drug enters the follicular orifice as opposed to penetrating via the continuous stratum corneum surface. Hence, the % follicular contribution refers to the flux of drug entering the follicular opening as a fraction of total transdermal drug flux. The skin sandwich system does not yield any data with regards to the fate of the drug once inside the follicle. Table 1 includes the relevant parameters for this study and all the others discussed below.

3.1. The influence of donor solution pH

The aim of these studies was to determine if varying donor solution pH within the physiological range (Sznitowska et al., 2001) affected hydrocortisone permeation into follicles. Fig. 2 shows the cumulative penetration versus time graphs for hydrocortisone buffered at pH values of 3 and 6. Fig. 3 shows the equivalent data for studies performed at pH values of 7.4 and 8.8. Each graph consists of two plots representing single epidermis experiments and sandwich experiments.

For the study run at pH 3, mean steady-state hydrocortisone flux across human epidermis was 29.54 ± 3.27 ng cm^{-2} h^{-1}, which is equivalent to a mean k_p of 0.94 × 10^{-4} cm h^{-1}. Mean steady-state hydrocortisone flux across sandwich membranes was 6.87 ± 0.19 ng cm^{-2} h^{-1}. This yields a J_{Sand}/J_{Ep} value of 0.23, corresponding to a follicular contribution to flux of 54%.

For transport studies conducted at pH 6, a mean single epidermal steady-state flux of 49.52 ng cm^{-2} h^{-1} was measured, corresponding to a mean k_p of 1.68 × 10^{-4} cm h^{-1}. For sandwich experiments, the mean steady-state flux was only 0.24 of that observed for the single membranes. This gives a follicular contribution to flux of 52%.

At pH 7.4, steady-state hydrocortisone flux was about 34 ng cm^{-2} h^{-1} for single epidermal membrane penetration. This yields a transepidermal permeability coefficient of 1.16 × 10^{-4} cm h^{-1}. This value is relatively close to the hydrocortisone permeability coefficient values measured by others such as 1.2 × 10^{-4} cm h^{-1} (Hadgraft and Ridout, 1987) and 2.3 × 10^{-4} cm h^{-1} (Johnson et al., 1995). In our studies, mean flux through sandwich membranes was about 9.20 ng cm^{-2} h^{-1} and this yields a J_{Sand}/J_{Ep} value of 0.27. This means that the follicular contribution was 46%.

For studies undertaken with a donor solution maintained at pH 8.8, mean steady-state flux for this drug through single epidermal membrane was 112.64 ng cm^{-2} h^{-1}. This corresponds to a mean k_p value of 3.82 × 10^{-4} cm h^{-1}. Mean steady-state hydrocortisone flux through SC/epidermal sandwiches was 31.47 ng cm^{-2} h^{-1}. Thus, the ratio of sandwich flux to epidermal flux was 0.28. This is equivalent to a follicular contribution of 44%.

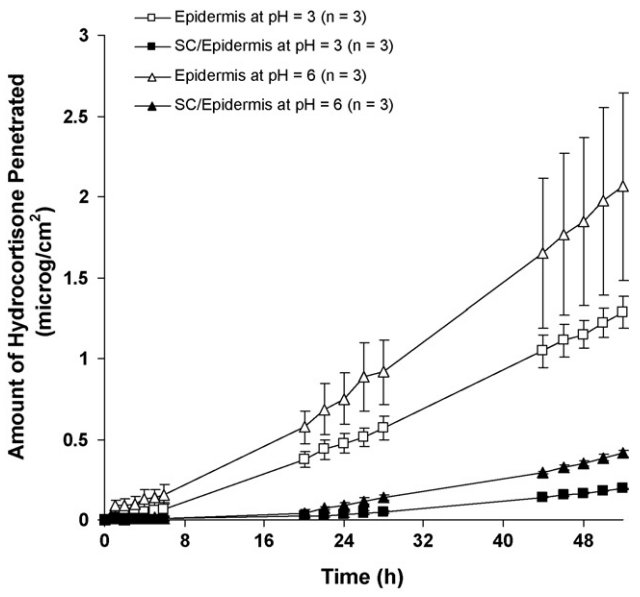

Fig. 2. Hydrocortisone penetration through both single epidermal and sandwich membranes with the donor solution maintained at pH3 or pH6. Error bars represent standard deviation values.

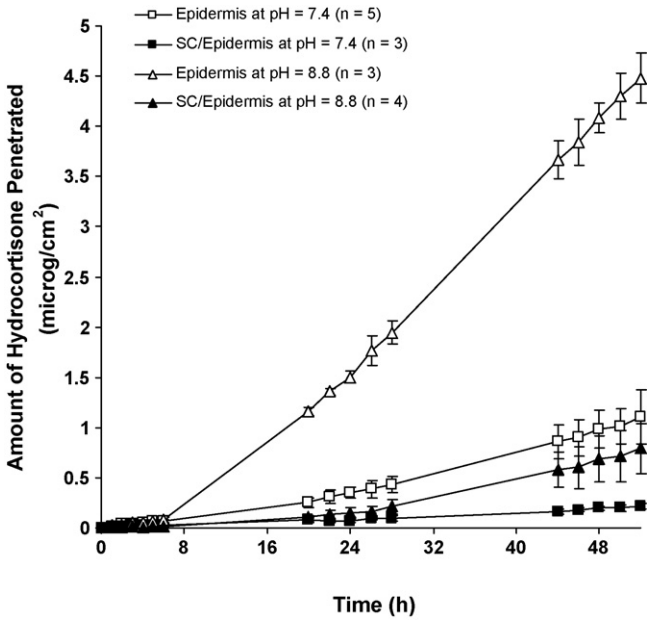

Fig. 3. Hydrocortisone penetration through both single epidermal and sandwich membranes with the donor solution maintained at pH7.4 or pH8.8. Error bars represent standard deviation values.

Thus, the results indicated that increasing the pH of the donor solution decreased the % follicular contribution of hydrocortisone flux. It is known that, normally, the stratum corneum surface is negatively charged due to the presence of fixed ionisable carboxylate and ammonium groups in this layer. One possible explanation is that making the vehicle progressively more alkaline will intensify the negative charges of the skin surface. The resulting electrostatic repulsion within the stratum corneum surface could conceivably distend the membrane, resulting in contraction of the follicular orifices. This should reduce the % follicular component of hydrocortisone flux. When considering this hypothesis, it should be remembered that the stratum corneum is somewhat elastic (Yuan and Verma, 2006) and that the follicular shunts constitute only a tiny fraction of the available surface area.

3.2. The influence of viscosity

Continuous shear viscosity measurements indicated that the apparent viscosity for the gel containing 0.6% (w/w) HPMC was 1.75 ± 0.05 P, whilst the apparent viscosity for the gel containing 1% (w/w) HPMC was 30.14 ± 0.01 P. Hot stage microscopy was employed to examine each gel formulation for the presence of crystals. The absence of birefringence in both gel formulations confirmed that hydrocortisone crystals were not present. Thus, in both HPMC gels, hydrocortisone concentrations were below saturation. Hence, the concentration of hydrocortisone in both gel formulations and the transepidermal permeability coefficients could be determined.

Fig. 4 presents the cumulative penetration versus time graph for the hydrocortisone-containing 0.6% (w/w) HPMC gel. The average flux through single epidermal membrane was only $6.54 \pm 2.63\ \text{ng cm}^{-2}\ \text{h}^{-1}$ which was equivalent to a mean k_p value of $0.22 \times 10^{-4}\ \text{cm h}^{-1}$. The mean steady-state hydrocortisone flux across SC/epidermal sandwiches was $3.02 \pm 1.42\ \text{ng cm}^{-2}\ \text{h}^{-1}$. Hence, the J_{Sand}/J_{Ep} value was 0.46, which corresponds to a follicular contribution of 8%.

For the 1% (w/w) HPMC gel (graph not shown), mean steady-state hydrocortisone flux across single and sandwich membranes was 9.74 and 4.08 ng cm^{-2} h^{-1}, respectively. This yielded a transepidermal permeability coefficient of $0.33 \times 10^{-4}\ \text{cm h}^{-1}$. The ratio of sandwich flux to epidermal flux was 0.42, translating to a follicular contribution of 16%.

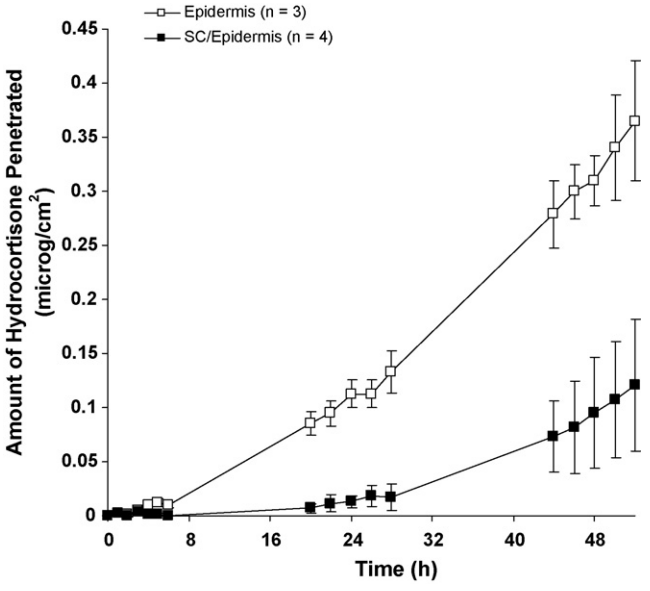

Fig. 4. Hydrocortisone penetration from 0.6% (w/v) HPMC gel across both single epidermal and sandwich membranes. Error bars represent standard deviation values.

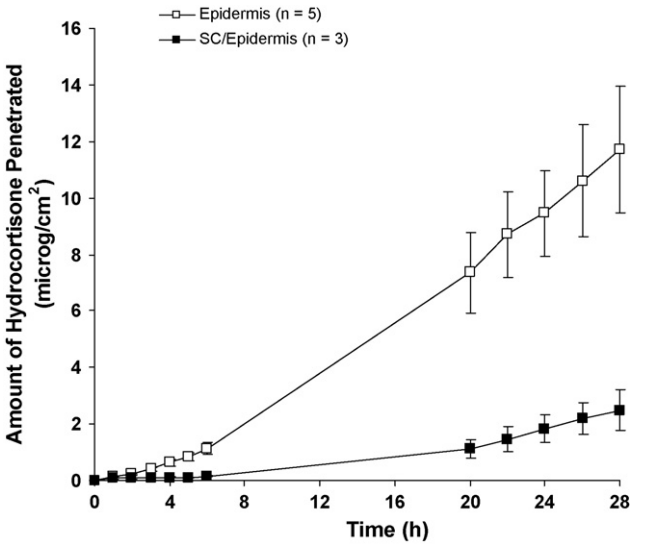

Fig. 5. Hydrocortisone penetration through both single epidermal and sandwich membranes pretreated with oleic acid. Error bars represent standard deviation values.

Hence, use of the gel formulations instead of an aqueous solution resulted in a much lower total drug flux. This may be due to the HPMC molecules hindering hydrocortisone diffusion through the gel and this process may be the rate-limited step in the permeation process. Moreover, when the topical formulation was a HPMC gel, proportionately less hydrocortisone entered through follicular orifices in comparison to when the formulation was an aqueous solution. This phenomenon could be due to the fact that unlike aqueous solution, these gels were sufficiently viscous so as to not flow into follicular orifices that are typically ~50–100 μm in diameter. Hence, a lack of direct contact between the drug formulation and skin surface at the orifice would impede intrafollicular penetration. With this explanation, the reason why the 1% (w/w) gel yielded a higher follicular component than the 0.6% (w/w) gel is unclear and requires further investigation.

3.3. The influence of chemical enhancers

Fig. 5 shows the hydrocortisone penetration data for skin tissue that was pretreated with oleic acid. Steady-state drug flux across human epidermis was $543.79 \pm 113.09\ \text{ng cm}^{-2}\ \text{h}^{-1}$, which is equivalent to a mean k_p of $18.46 \times 10^{-4}\ \text{cm h}^{-1}$. Steady-state drug flux across sandwich membranes was $184.05 \pm 44.82\ \text{ng cm}^{-2}\ \text{h}^{-1}$. This constitutes a J_{Sand}/J_{Ep} value of 0.34, which translates to a follicular component of 32%.

With regards to skin pretreated with menthone (Fig. 6), steady-state hydrocortisone flux across epidermal and sandwich membranes were approximately 39 and 14.85 ng cm^{-2} h^{-1} respectively. This yielded a mean transepidermal permeability coefficient of $1.32 \times 10^{-4}\ \text{cm h}^{-1}$. The J_{Sand}/J_{Ep} value is 0.38 and this is equivalent to a follicular contribution to flux of 24%.

When human skin was pretreated with carvone, mean steady-state hydrocortisone flux across single epidermal samples was $27.42 \pm 3.19\ \text{ng cm}^{-2}\ \text{h}^{-1}$. This corresponded to a mean k_p of $0.93 \times 10^{-4}\ \text{cm h}^{-1}$. The curves are shown in Fig. 7. In the sandwich experiments, the mean steady-state flux was 0.55 of that observed for the single membranes. This corresponds to a % follicular contribution of −10% which is obviously illogical. If steady-state flux through the sandwich was half that through the single epidermis,

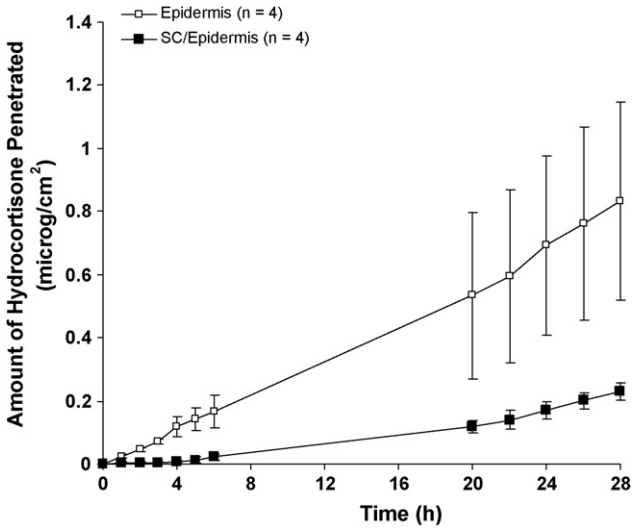

Fig. 6. Hydrocortisone penetration through both single epidermal and sandwich membranes pretreated with menthone. Error bars represent standard deviation values.

the follicular contribution would be zero. The fact that it is slightly more than half (0.55) suggests that some aspect of the experimental protocol is causing chemical enhancer concentration in the sandwich to be greater than that in the single membrane. One possible explanation is that tissue exposures to carvone in propylene glycol followed by the two subsequent hydration steps (see Sections 2.4 and 2.5) result in the final sandwiches retaining proportionally greater enhancer quantities than the single epidermal membranes. Such a process would account for the apparent negative follicular contribution.

Fig. 8 presents the permeation data derived in the case of SLS pretreatment. Mean steady-state hydrocortisone flux across single epidermal tissue and sandwich membranes was about 43 and 24 ng cm^{-2} h^{-1}, respectively. This yielded a transepidermal permeability coefficient of 1.47×10^{-4} cm h^{-1} and a J_{Sand}/J_{Ep} value of 0.56. This corresponds to a follicular component of −12%. Again, the same experimental artifact described above for carvone is probably responsible for this negative value.

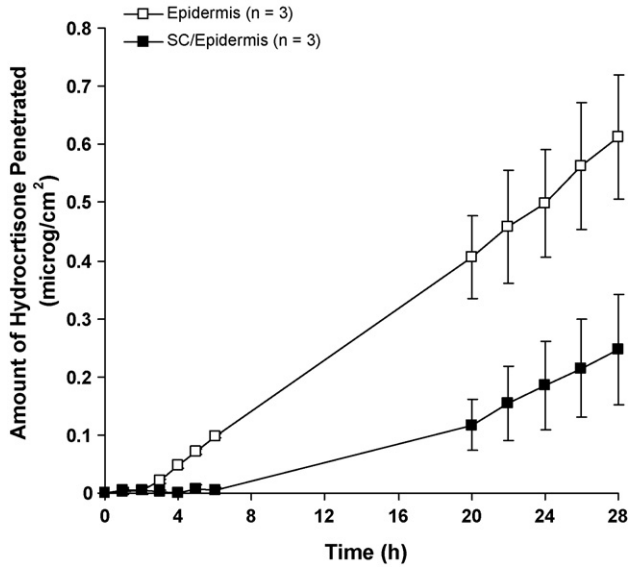

Fig. 7. Hydrocortisone penetration through both single epidermal and sandwich membranes pretreated with carvone. Error bars represent standard deviation values.

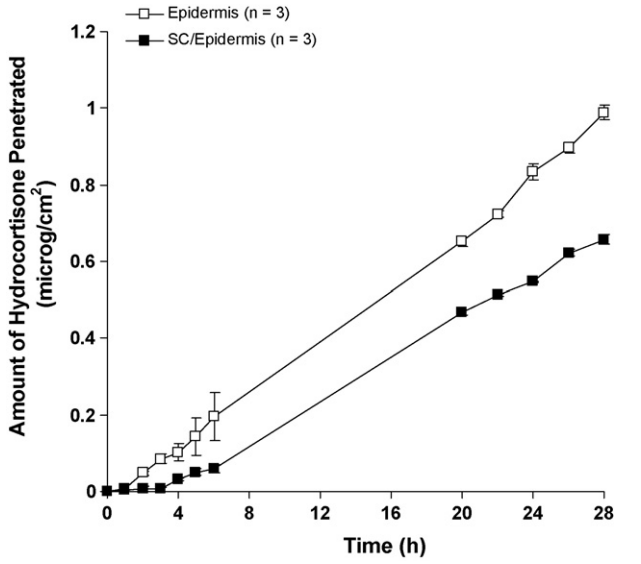

Fig. 8. Hydrocortisone penetration through both single epidermal and sandwich membranes pretreated with sodium lauryl sulphate. Error bars represent standard deviation values.

These findings indicate that pretreating skin samples with all four chemical enhancers reduced the subsequent intrafollicular penetration of hydrocortisone. This makes sense given the fact that chemical enhancers will permeabilise the continuous stratum corneum, thus making entry via follicles less quantitatively significant. However, negative follicular contribution values for two of the enhancers indicate that another experimental artifact was influencing the results.

3.4. The influence of skin hydration

One critique of the skin sandwich approach is that it involves employing skin samples that are much more hydrated than normal skin under regular clinical conditions. With this in mind, a set of experiments were undertaken in order to determine if using a much briefer hydration period of 12 h instead of 48 h (see Section 2.5) would affect the results obtained.

Fig. 9 presents the hydrocortisone flux plots for epidermal and sandwich membranes – each both fully hydrated and partially hydrated. Steady-state hydrocortisone flux across partially hydrated human epidermis was 50.69 ± 4.09 ng cm^{-2} h^{-1}, corresponding to a mean k_p of 1.72 cm h^{-1}. Mean steady-state hydrocortisone flux through partially hydrated SC/epidermal sandwiches was 9.82 ± 3.23 ng cm^{-2} h^{-1}. Thus, when using the partial hydration protocol, J_{Sand}/J_{Ep} was 0.19 and the % follicular contribution was 62%. This is higher than the follicular contribution value of 46% that was obtained when using the full hydration protocol.

In order to identify the perturbations involved, it is necessary to compare steroid flux across fully hydrated sandwiches with steroid flux across partially hydrated sandwiches. Here, no statistically significant difference was found ($P > 0.05$). However, there was a significant difference between solute flux across fully hydrated single membranes and solute flux across partially hydrated single membranes ($P < 0.05$). In practice, sandwich membranes represent 'follicle-free' continuous stratum corneum. Since hydration does not appear to affect solute flux across continuous skin, the presence of follicles in the single epidermal membranes must be responsible for the observed difference in the solute permeation rate.

It is known that hydration can increase the thickness of the stratum corneum several fold because binding of water within the intracellular keratin results in the swelling of the cells in the skin.

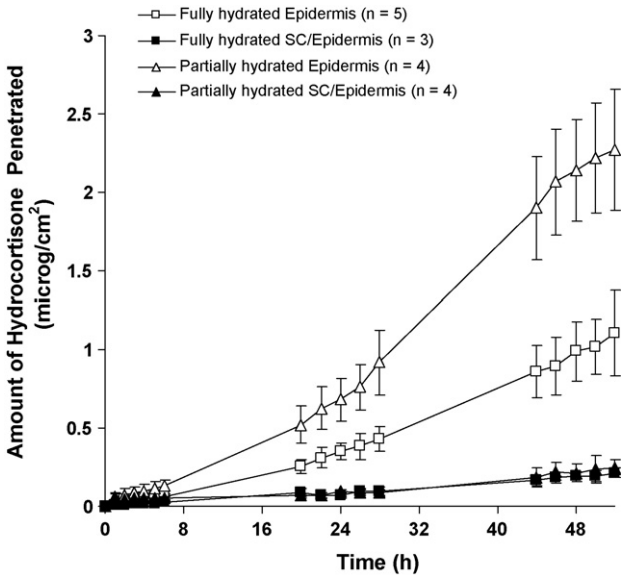

Fig. 9. Hydrocortisone penetration through both fully-hydrated and partially hydrated skin membranes. Error bars represent standard deviation values.

Swelling of the continuous stratum corneum has been reported to partially close the follicular shunts (Peck et al., 1994; El Maghraby et al., 2001, 2006). This explains why the % follicular contribution is much higher when partially hydrated epidermal membranes were used compared to fully hydrated epidermal membranes.

Thus, this attempt to modify the skin sandwich technique itself through use of a briefer hydration protocol was unsuccessful. The main problem is that the stratum corneum swells with continuing water uptake, in an asymptotic manner. The sandwich method depends upon stratum corneum dimensions being fixed, which occurs when the upper limit to swelling is more or less attained (Barry, 2002). A 4-fold shortening of the hydration period not only confounds this assumption in theoretical terms but it also makes a practical difference in terms of the data obtained.

4. Conclusions

Our skin sandwich studies, involving a saturated aqueous solution, have shown that the follicular contribution to hydrocortisone flux slightly decreases with increasing alkalinity in the pH range 3–8.8. Substituting the aqueous solution with aqueous gels resulted in a marked reduction in intrafollicular permeation. Pretreating the skin with chemical enhancers also reduced the follicular contribution to flux, probably mainly due to permeabilisation of the continuous stratum corneum. Furthermore, it was not possible to satisfactorily modify the skin sandwich method so that it could be deployed on less hydrated skin. For future work, it would be interesting to test the influence of formulation pH, viscosity and chemical enhancers on intrafollicular delivery using other recently emergent hair follicle models.

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