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RESEARCH PAPER

Assessment of the Lateral Diffusion and Penetration of Topically Applied Drugs in Humans Using a Novel Concentric Tape Stripping Design

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ABSTRACT

Purpose To determine the extent of lateral spread and stratum corneum (SC) penetration of caffeine (CAF), hydrocortisone (HC) and ibuprofen (IBU) using a novel concentric tape stripping technique.

Method Ethanolic solutions of CAF, HC or IBU were applied to the forearm of 8 volunteers. At various time points, 10 successive layers of SC were removed by stripping with tapes perforated into concentric rings and analysed for drug concentration and mass of SC protein. *In vitro* permeation studies assessed the percutaneous absorption of these compounds across human skin.

Results CAF and IBU showed significant lateral spreading across the SC while HC formed a drug depot at the site of application. Relative to the applied dose, the *in vivo* recovery of all compounds from the combined 10 strips at 3 mins ranged between 83.0 and 92.9 % and decreased to between 64.5 and 66.9 % at 3 h. IBU recovery further decreased to 47.7 ± 5.6 % at 6 h, correlating with greater *in vitro* penetration relative to CAF and HC.

Conclusion Drug concentration decreased with increased lateral distance from the application site. The lower recovery of IBU in the upper tape strip regions compared to CAF and HC may be a consequence of greater penetration into the SC with time.

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INTRODUCTION

Transdermal and topical drug delivery are methods by which an active drug is applied onto the skin surface for the purpose of systemic or local treatment, respectively. For a transdermally or topically applied drug to become bioavailable, it must follow a tortuous route of penetration around the corneocytes and through the interceulluar lipid domains of the stratum corneum (SC) (1). In this respect, the inherent barrier function of the SC limits the usefulness of this route of delivery.

It is generally accepted that the bioavailability of topically applied drugs is in the order of 5–10 % (2) and in many instances, where the formulation is non-occlusive, this value is even lower. It is usually assumed that the non-bioavailable portion of a dose of drug applied to the skin in a non-occlusive manner is either metabolized, shed via epidermal desquamation or washed or rubbed off. To further complicate the situation it is also feasible that drug may spread across the skin surface outside the original area of application. Only a few studies have examined the process of lateral drug diffusion that occurs in tandem with the process of drug penetration into the skin (3–6). Nevertheless, the exact distance that drugs spread radially from their application site, as well as the extent of their lateral diffusion within the skin bilayers, have been poorly defined. The importance of better understanding the fate of drugs applied to the skin is clear. Recent 'black box' warnings placed on some topically applied formulations warn of documented cases of secondary systemic exposure caused by contact with the skin of patients using such products (7). There is therefore an

interest in this area within regulatory bodies such as the US FDA.

A drug that may not readily partition into and through the SC may be more inclined to spread radially across the upper most regions of the SC, while a drug that readily penetrates through the SC may retain the majority of drug within close radial proximity to the application site and therefore have a lower tendency to undergo lateral diffusion. While the effect of physicochemical properties on the penetration behaviour of drugs is widely understood, less attention has been given to appreciating the impact of physicochemical properties on lateral diffusion across the skin. Lateral diffusion is a possible route of drug loss and thus, in order to gain a better understanding of the fate of unabsorbed drugs, the influence of the physicochemical properties on the simultaneous process of penetration in the upper layers of the SC and lateral spreading is required.

Caffeine (CAF), hydrocortisone (HC) and ibuprofen (IBU) are three model compounds with different physicochemical properties, which may cause them to exhibit different degrees of penetration and spread across the SC. It has been reported that the logP and molecular weight of a permeant are significant predictors of drug penetration across the skin (8). CAF is very hydrophilic with a logP of −0.07 (9); IBU is deemed to have an ideal logP for skin penetration of 3.51 (10) while HC has an intermediate logP of 1.43 (11). CAF and IBU have comparable molecular weights of 194.19 g/mol and 206.28 g/mol, respectively, while HC has a larger a molecular weight of 362.46 g/mol which may impact the spreading and penetration behaviour of HC.

While it has been demonstrated that these three model compounds can penetrate the SC (12–17), literature relating to the competing processes of lateral diffusion and penetration of CAF, HC and IBU (or other molecules with varied physicochemical properties) are scarce. Most studies that evaluate the penetration of permeant across the skin using tape stripping ignore the need to determine the lateral diffusion of the permeant. Conversely, some studies that have utilised tape stripping to study lateral diffusion have not specified the distance to which a drug may spread. Taken together, no studies have successfully followed both the penetration and lateral diffusion behaviour of drugs across the skin concurrently. However, the need to determine both the penetration and lateral diffusion fate of drugs is essential to understand the risks of secondary exposure to third-parties caused by contact of the skin area beyond the site of application. It has been reported that the rate of lateral diffusion is considerably faster than that of transbilayer transport over equivalent distances (18,19), and so it would be predicted that lateral diffusion is a significant contributor to drug loss following application. Following topical application, lateral diffusion of a permeant can occur on the surface of the SC, along the plane of the lipid bilayers within the SC as well transporting from one bilayer in a multibilayer assembly into a parallel bilayer following diffusion through the adjacent lipid channels between corneocytes (as shown in Fig. 1). Thus, the process of lateral diffusion may play an important role in the mechanism of solute transport through the SC.

Therefore, the aim of this study was to determine the *in vivo* lateral diffusion and penetration behaviour of three model drugs with varying lipophilicities, across the SC of humans by utilising specially designed perforated concentric adhesive tapes. The concentric adhesive tapes were custom made for these studies and their ability to remove topically applied compounds and SC protein were validated to ensure the simultaneous assessment of lateral diffusion and penetration behaviour of topically applied compounds. *In vitro* permeation studies were also carried out to assist in the interpretation of the *in vivo* human studies undertaken with this novel tape stripping technique.

MATERIALS AND METHODS

Materials

CAF, HC, IBU, bovine serum albumin (BSA), Bradford reagent, sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were purchased from Sigma-Aldrich (Castle Hill, New South Wales, Australia). Ethanol (EtOH), methanol (MeOH), sodium hydroxide (NaOH), hydrochloric acid (36 %) (HCl) and phosphoric acid were obtained from Merck (Kilsyth, Victoria, Australia). Potassium dihydrogen orthophosphate was acquired from Univar (Ingleburn, New South Wales, Australia) and purified water was obtained from a Milli-Q™ water purification system (Millipore, Bedford, MA, USA). Ethoxy 20 oleyl alcohol (VOLPO N20) was purchased from Croda (Wetherill Park, New South Wales, Australia).

Human Subjects

Eight healthy volunteers (4 males and 4 females) provided written consent to participate in the study which was approved by the Standing Committee on Ethics in Research involving Humans (SCERH), Monash University, Australia (Project #CF08/1125–2008000555). The participants were aged between 24 and 37 years of age and had no history of skin disease. Each participant was asked to refrain from applying any topical medicaments to their left and right flexor forearms at least 48 h prior to an experiment.

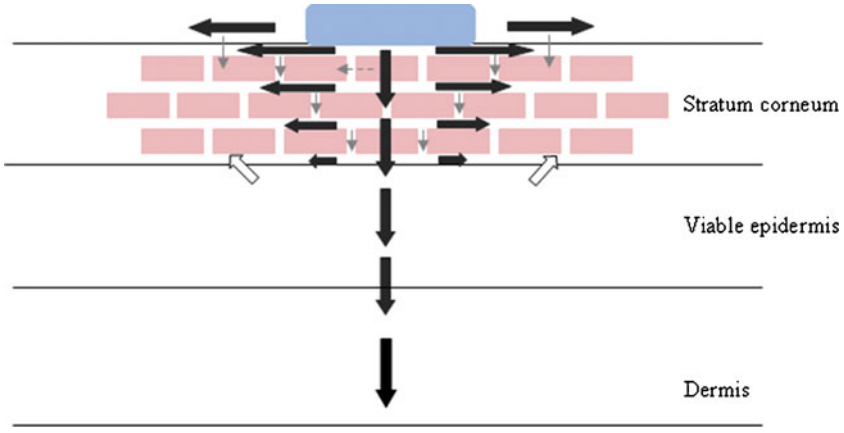

Fig. 1 Routes of drug penetration and lateral diffusion across the skin barrier (not drawn to scale).

Topical Application

The volar forearm of each participant was wiped with Kimwipes™ saturated with EtOH to remove any sebaceous lipids or contaminants on the skin surface. Circles of 0.5 cm^2 were marked on the flattest plane of the left and right volar forearms to clearly outline the application area. An area of 0.5 cm^2 was chosen to replicate the area of the innermost circle of the concentric adhesive tapes used in these studies. The sampling site was also clearly marked with permanent marker prior to dosing the skin to ensure each tape strip removed SC from the same site in a manner that did not interfere with the lateral spreading. The participant extended both forearms over a work bench with the volar forearm facing upwards. A 1.8 μL aliquot of an ethanolic solution containing either 0.8 % w/v of CAF, 2 % w/v of HC, or 5 % w/v of IBU was dosed onto individual marked areas of the skin. The concentration of CAF and HC was chosen to reflect 80–85 % of their saturated solubility concentration while the concentration of IBU was chosen to reflect that of usual topical formulations. The forearm remained flat and rested on the bench for approximately 30 s after dosing to allow for solvent evaporation. The solutions were allowed to remain in contact with the skin for 3 mins, 3 h and 6 h prior to tape stripping. During these sampling time points, the solutions were left unoccluded and the participant was required to wear a short-sleeved shirt or have their sleeves rolled up to avoid loss of drug through being rubbed off onto clothing. The participants were allowed to resume their daily activities, although were advised to avoid activities that would cause perspiration as this may alter the spreading and/or penetration behaviour of the drugs applied.

Tape Stripping

Tape stripping was performed using custom designed concentric tape perforated into 4 sections (3 M, Product No 8440CONRING32), as shown in Fig. 2. The innermost segment (section 1) represents the initial application site and has a diameter of 8 mm. Section 2 forms the first ring around the application area and has an outer diameter of 16 mm. The third ring has an outer diameter of 24 mm and the largest ring has an outer diameter of 32 mm. The size of the concentric tape was selected based on preliminary data obtained in our laboratory which showed the potential distance of spread following drug application over an initial area of 0.5 cm^2.

Each circular section on the adhesive tape can be separated along the perforated lines into individual segments, allowing for the amount of drug and SC removed from each tape section to be quantified individually. In addition, the depth of drug penetration and the extent of lateral diffusion within the SC layers can also be determined concurrently by cutting the concentric tape into two equal halves as shown in Fig. 2. One half of the concentric tapes were further separated along the perforated lines into individual segments to quantify for the amount of drug removed, while the other half of the concentric tapes were split into individual sections to quantify for the amount of SC protein removed using the Bradford assay as discussed below.

At 3 mins, 3 h and 6 h after application, the concentric tapes were transferred to the marked skin area with tweezers, ensuring that section 1 of the concentric tapes was placed directly over the pre-marked application area. A stainless steel slab weighing 1200 g and measuring 2.8×6×9 cm was placed over the tape for 3 s to ensure even pressure was applied across the tape. The tape was then removed from the SC surface and split into two equal halves (for drug and SC protein quantification). 10 sequential tape strips were removed at each corresponding sample area at each time point.

Quantification of Model Drug

To quantify the amount of drug removed, the perforated ring segments from one half of the divided tape were further split into their individual sections, as shown in Fig. 2 and submerged in individual plastic vials containing 10 mL of extraction

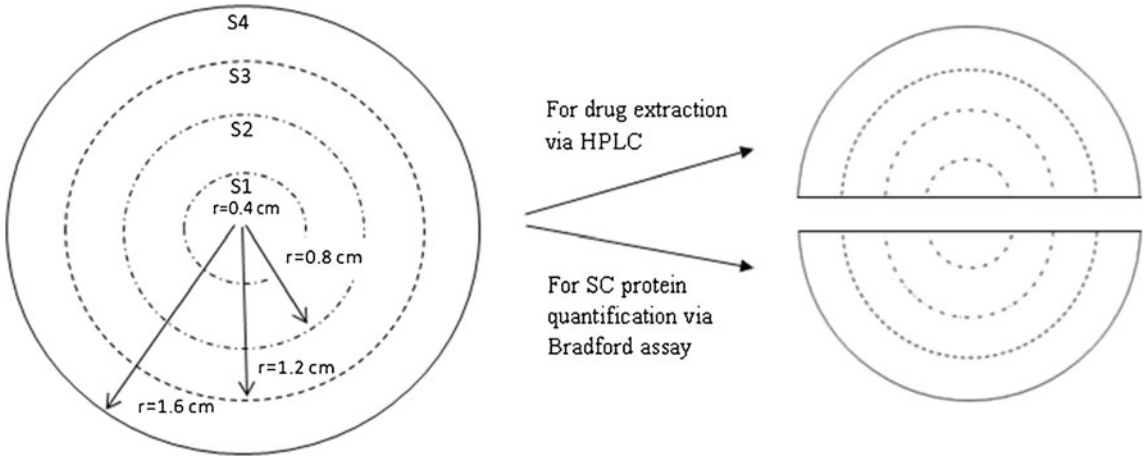

Fig. 2 Schematic diagram of the novel concentric tape system and the post-strip splitting of the concentric tape for drug extraction and SC protein quantification.

solvent. The extraction solvent for CAF, HC and IBU consisted of MeOH:H_2O (50:50), MeOH:H_2O (60:40) and MeOH:HCl 36 % v/v (90:10), respectively. Vials containing adhesive tape obtained from CAF and HC application were sonicated for 1 h. For samples containing adhesive tape obtained following IBU application, vials were incubated in a shaking water bath set at 80 strokes per min at 60 °C for 30 mins prior to sonicating. The amount of drug extracted from each tape strip was analysed using validated HPLC/UV and HPLC-fluorescence methods as described in Table I. Validation of the CAF, HC and IBU extraction procedures was conducted by spiking untreated adhesive tape with known amounts of drug in solution (at low, intermediate and high concentrations of the expected concentration range) and peak areas compared by HPLC to solutions of each drug prepared in the absence of adhesive tape. Using the validated extraction procedure, between 91.5 and 103.4 % of CAF, HC and IBU, respectively, was recovered for all test concentrations.

Quantification of SC Protein

It is important to normalize the amount of drug extracted from each tape strip to the corresponding mass of SC protein from the same tape strip in order to follow the

Table I Chromatographic Conditions used to Quantify the Amount of CAF, HC and IBU Extracted from Adhesive Tape Following *In Vivo* Application to Humans

	Drug		
Condition	CAF	HC	IBU
Mobile phase	Solvent A: 0.057 % v/v phosphoric acid Solvent B: MeOH	Solvent A: MeOH Solvent B: H_2O	Solvent A: 5 mM phosphate buffer (pH 2.4[a]) Solvent B: MeOH
Flow method	Gradient[b]	Gradient[c]	Gradient[d]
Flow rate	1 mL/min	1 mL/min	1 mL/min
Injection volume	50 μL	100 μL	10 μL
UV detection	273 nm	245 nm	–
Fluorescence detection	–	–	Excitation λ: 263 nm Emission λ: 288 nm
Column temperature	Ambient	Ambient	40 °C
Run time	20 mins	17 mins	21 mins
Retention time	~6.6 mins	~3.9 mins	8.7 mins
Concentration range	0.01–1.75 μg/mL	8.75–875 ng/mL	0.125–12.5 μg/mL

[a] pH adjusted with 1 M phosphoric acid

[b] 0–9 mins, 22 % B; 9–10 mins, 22–37 % B; 10–17 mins, 37 % B; 17–18 mins, 37–22 % B; 18–20 mins, 22 % B

[c] 0–5 mins, 40 % B; 5–6 mins, 40–10 % B; 6–13 mins, 10 % B; 13–14 mins, 10–40 % B; 14–17 mins, 40 % B

[d] 0–10.5 mins, 75 % B; 10.5–11.5 mins, 75–85 % B; 11.5–17 mins, 85 % B; 17–18 mins, 85–75 % B; 18–21 mins, 75 % B

drug-depth *versus* penetration behaviour of a permeant. We have adopted a modified Bradford assay (20) using lyophilized bovine serum albumin as standards to evaluate the mass of SC protein removed per tape strip. Following tape strip removal from the SC, the remaining half of the divided tape was separated into individual tape sections and immersed into 12-well plates containing 700 µL of NaOH in each well. The plates were incubated for 1 h at 22 °C on a horizontal plate using an Eppendorf Thermo-mixer Comfort (Hamburg, Germany) at 300 rpm. Thereafter, a 50 µL aliquot from each well was transferred into a 96-well plate and 250 µL of Bradford reagent added. The plates were then securely wrapped in aluminium foil to protect them from light and incubated at ambient conditions on the bench for 30 mins. Each plate was then placed in a FLUOstar Optima (BMG Labtechnologies, Offenburg, Germany) and absorbance measured at 595 nm. The mass of SC protein removed from each adhesive tape section was calculated from the determined absorbance maxima at 595 nm on the basis of a calibration curve prepared from BSA standard solutions (100–600 µg/mL).

In Vitro Skin Membrane

Surgically excised samples of skin from one male and one female were obtained after abdominoplastic surgery with informed consent and approval from the SCERH, Monash University, Australia (Project #2006/565). Full-thickness skin (with subcutaneous fat attached) was defrosted for 3–4 h, the skin was rinsed by cleaning the surface with MilliQ water and the surface was swabbed with paper towels. Dermatomed skin was then prepared using a dermatome slicer set to cut at a thickness of 500 µm (Humeca, Netherlands). The separated skin was then immersed in cold water for 30 mins and rinsed 3 times until the water appeared clear. The skin membranes were then transferred onto aluminium foil and stored in a freezer until required.

Permeation Studies

In vitro skin diffusion studies were carried out using stainless steel flow-through diffusion cells maintained at 32 °C by the continuous pumping of thermostated water through hollow stainless steel bars supporting the cells. Prior to mounting the skin over the receptor chamber, the chamber was filled with receptor solution (isotonic phosphate buffer pH 7.4 for CAF and IBU; 0.5 % w/v VOLPO N20 in water for HC) and a wire mesh was placed into the receptor chamber. The wire mesh was incorporated to promote turbulent flow of receptor solution through the receptor well, which alleviates the formation of unstirred, limiting boundary layers and thus aids in the maintenance of sink conditions, as well as to prevent the formation of bubbles beneath the skin.

Dermatomed skin (500 µm) that was previously cut into square pieces was mounted in specially designed diffusion cells with a large donor chamber area of 3 cm^2. The donor chamber was then filled with approximately 2 mL of MilliQ water or until a convex water meniscus was formed and left for 30 mins to assess skin integrity. If the convex water meniscus at the end of the 30 min period appeared unchanged, the integrity of the skin was assumed intact and therefore suitable to be used in the permeation study. The water was removed from the donor chamber and the receptor solution was pumped through the receptor chamber for a further 10 mins at a flow rate of 0.5 mL/hr using a peristaltic pump (Watson Marlow Microcasette Pump, UK). In total, the skin was equilibrated with the receptor fluid for ~45 mins before a 1.8 µL dose of donor solution was applied. The donor solutions consisted of CAF 0.8 % w/v, HC 2 % w/v or IBU 5 % w/v dissolved in EtOH. The diffusion experiments were carried for a period of 6 h, whereby the receptor fluid was collected into vials every 30 mins for the first 3 h, then a final sample collected at 6 h, using an automated fraction collector (ISCO Retriever II, NE). The amount of drug permeated over time was quantified using validated HPLC assays as shown in Table II.

Analytical Method

Unknown concentrations of CAF, HC and IBU extracted from adhesive tapes and *in vitro* permeation studies were determined using HPLC with a Water Symmetry® C_{18} column (5 µm particle size, 3.9×150 mm internal diameter) equipped with a Waters Symmetry® C_{18} guard column (3.9×20 mm) (Waters, Milford, MA). The HPLC system used for CAF and HC quantification from adhesive tape and to measure the *in vitro* permeation of CAF, HC and IBU across human skin consisted of a Waters 2690 separations module, Waters 2487 dual wavelength absorbance detector, Waters 610 pump, Waters 600E system controller and a Waters 712 autosampler. The HPLC system used for IBU quantification from adhesive tape consisted of a Prominence CBM-20A communications module equipped with a Prominence LC-20AD separations module, Shimadzu RU-10AXL fluorescence detector, Prominence SIL-20A HT autosampler and a Prominence CTO-20A column oven. The chromatographic conditions for drug quantification from adhesive tape are detailed in Table I and the corresponding conditions for *in vitro* studies are outlined in Table II. The method of linear regression (weighted by a factor of 1/×) which achieved a correlation coefficient (r^2) of at least 0.99 was used to determine the relationship between the mass of drug extracted and the peak area of drugs detected by HPLC quantification.

Table II Chromatographic Conditions used to Quantify the Amount of CAF, HC and IBU Permeating Dermatomed Human Skin *In Vitro*

	Drug		
Condition	CAF	HC	IBU
Mobile phase	0.05 % v/v TFA in 10 % ACN in MilliQ water:MeOH (85:15)	MilliQ water:MeOH:ACN (58:27:15)	Line A: MilliQ water pH 2.5[a] Line B: ACN Gradient[b]
Flow rate	1.1 mL/min	1.0 mL/min	1.0 mL/min
Injection volume	30 μL	100 μL	100 μL
UV detection	272 nm	245 nm	219 nm
Column temperature	35 °C	40 °C	30 °C
Run time	6 mins	9 mins	9 mins
Retention time	4.0 mins	5.6 mins	3.3 mins
Concentration range (μg/mL)	0.1–50	0.03–0.5	0.02–5

[a] pH adjusted to with 1 M phosphoric acid

[b] Gradient run: 0–0.5 mins, 40 % B; 0.5–2 mins, 40–90 % B; 2–3 mins, 90 % B; 3–4 mins, 90–40 % B; 4–9 mins, 40 % B

Data Analysis

The amount of drug removed from each concentric tape section was normalised against the mass of SC protein removed by the same concentric tape section to account for inter-individual differences in SC removal. The normalised concentration of drug was plotted against the cumulative weight of SC protein per unit area. To allow for the easy visualisation of both lateral diffusion and penetration across the SC, the normalised data were schematically represented on contour plots (SigmaPlot 11 for Windows). The percentage recovery of CAF, HC and IBU at 3 mins, 3 h and 6 h relative to the applied dose was calculated in order to evaluate whether any difference in recovery with time may be a result of further penetration into the skin. In the following sections, the distance of drug spread from the application site will be referenced as section 1, section 2, section 3 and section 4, which equates to distances of between 0–4 mm, 4–8 mm, 8–12 mm and 12–16 mm, respectively (as shown in Fig. 2).

Statistical Analysis

Statistical analysis was carried out using IBM SPSS Statistics 19 for Windows. In order to compare significant differences between the recovery of CAF, HC and IBU over time, as well as to determine whether there was a significant difference in the percentage of each drug recovered per unit area in each concentric ring, a one way repeated measures analysis of various (ANOVA) test was used. A probability of $p<0.05$ was deemed significant. All data are presented as mean ± SEM ($n=8$), unless stated otherwise.

RESULTS

Lateral Spreading and Penetration of CAF, HC and IBU Across Human Skin *In Vivo*

Figure 3 gives a two-dimensional view of the distance of spread of CAF, HC and IBU on the surface of the SC and within the SC *versus* the depth of penetration at 3 mins, 3 h and 6 h after application to humans. A higher colour intensity indicates a higher drug concentration, according to the colour legend. However, it should be noted that given the differences in the maximum intensities for each drug (due to the different concentration of each drug dosed), absolute comparison in amount of drug that has spread or penetrated should not be made between CAF, HC and IBU.

At 3 mins after application, all marker drugs formed a reservoir in the superficial layers of the skin as shown in Fig. 3a, d and g. CAF formed a relatively flat reservoir that spread over the upper skin layers, while HC formed a deep depot mainly concentrated within the lateral boundaries of section 1. IBU generally formed both a deeper and wider reservoir than that exhibited by CAF and HC. The recovery of CAF decreased over 6 h as demonstrated by the reduction in colour intensity of the contour plots shown in Fig. 3a–c. The loss of CAF, however, was not reflected by further penetration into the underlying skin layers or increased lateral spread. Similar observations were apparent for HC as seen in Fig. 3d–f. In contrast, the amount of IBU detected between 400 and 600 μg/area depth of the SC appeared to increase in section 3 between 3 h and 6 h, as shown in Fig. 3h–i. However, it cannot be deduced whether this increase in IBU within the SC was due to lateral diffusion within the SC bilayers or penetration.

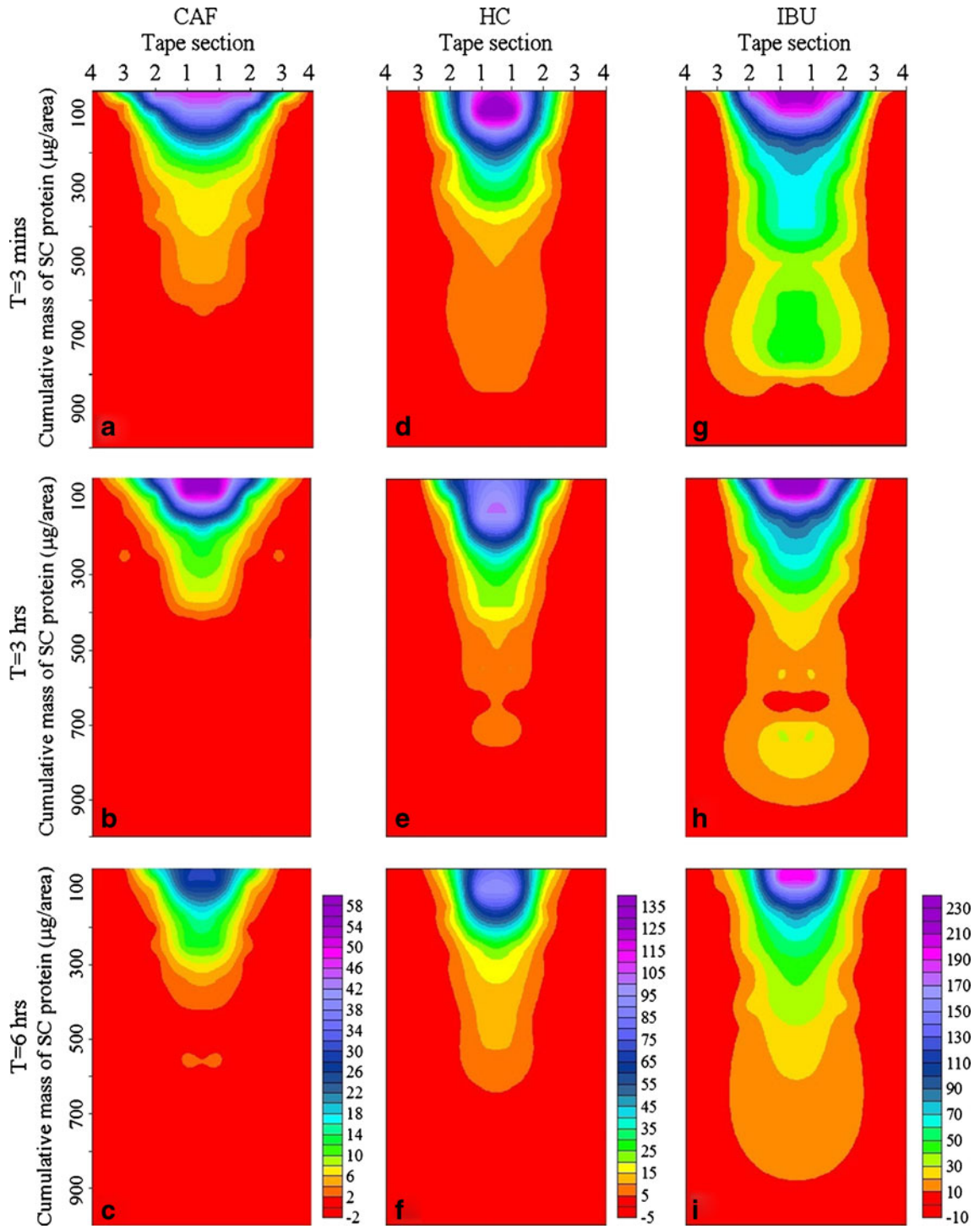

Fig. 3 Contour plots displaying the extent of lateral spread and penetration of CAF, HC and IBU at 3 mins, 3 h and 6 h after topical application to humans (mean, $n=8$). Legend values are expressed as concentration of drug (ng/µg of SC protein).

Upon closer inspection of Fig. 3, it is also interesting to note that at sites directly beneath the application area (section 1), CAF, HC and IBU penetrated to differing depths of the SC. CAF could only be detected to a depth of approximately 600 µg/area, while HC could be detected up to a depth of ~800 µg/area and IBU penetrated the greatest depth to approximately 1000 µg/area before no drug could be detected. These results may further attest the influence of drug lipophilicity on penetration as it was verified (in the following section) that the type of drug applied does not affect the amount of SC protein removed by tape stripping.

The lateral spreading behaviour of CAF, HC and IBU was further supported by Fig. 4a–c. At 3 mins, the normalized concentration of CAF (Fig. 4a) and IBU (Fig. 4b) in section 2 was similar to that detected in the application area (section 1), while the concentration of HC (Fig. 4c) in section 2 of each subsequent tape strip is less than half the concentration detected in section 1 of the corresponding tape strip.

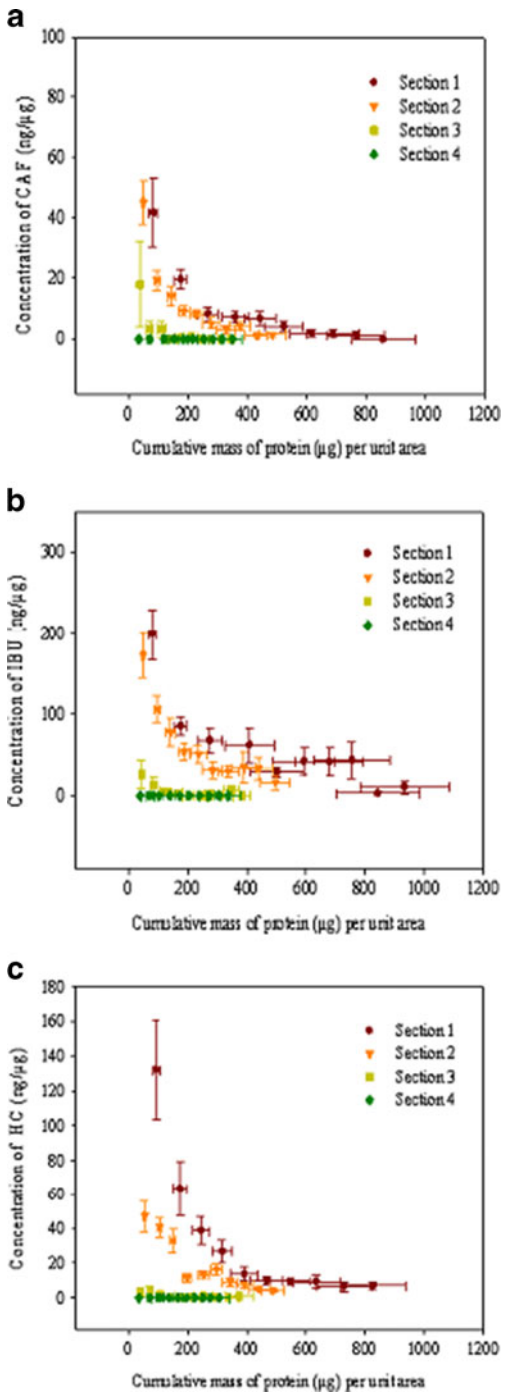

Fig. 4 Distribution profile of (**a**) CAF (**b**) IBU and (**c**) HC across the SC *in vivo* at 3 mins after application to humans (mean ± SEM, $n=8$).

This suggests that CAF and IBU undergo a relatively rapid process of lateral diffusion both on the surface of the SC and within the SC and display a higher tendency to spread than HC.

As can also be seen in Fig. 4a–c, all permeants show a common trend whereby the concentration of CAF, HC and IBU removed was highest in the first tape strip and progressively decreased with increased SC depth. Small amounts of CAF, HC and IBU were detected in section 3 at 3 mins after application but negligible amounts detected in section 4.

Recovery of CAF, HC and IBU from Adhesive Tape *In Vivo*

The recovery of CAF, HC and IBU extracted from all 4 sections of the concentric tapes of the combined 10 tape strips relative to the applied dose was measured to assess whether any differences in recovery (between drugs) may be attributable to their different physicochemical properties. The percentage recovery of CAF, HC and IBU from the combined 10 tape strips at 3 mins, 3 h and 6 h relative to the applied dose is displayed in Table III. While there was a significant reduction in the recovery of all 3 drugs at 3 h relative to the applied dose, a significant difference between the recovery at 3 h and 6 h was only evident for IBU, suggesting a progressive depletion of IBU from the skin surface.

Effect of Drug on SC Protein Removal Using Adhesive Tape

As the concentration of CAF, HC and IBU removed per tape strip was normalised against the mass of SC protein removed, it was of interest to assess whether the selection of drugs influenced the amount of SC protein removed with adhesive tape and additionally, whether the duration of drug-to-skin contact affected SC protein removal. Figure 5 presents the cumulative mass of SC protein removed per unit area from skin sites under sections 1, 2, 3, 4 of the

Table III Percentage Recovery of CAF, HC and IBU (relative to the applied dose) at 3 mins, 3 h and 6 h after Application to Humans (mean ± SEM, $n=8$)

	% Recovery		
Time	CAF	HC	IBU
3 mins	92.9 ± 5.6	83.0 ± 6.8	86.6 ± 5.6
3 h	64.5 ± 9.4[a]	66.9 ± 8.2[a]	66.2 ± 3.7[a]
6 h	53.2 ± 9.9[a]	64.2 ± 9.8[a]	47.7 ± 5.6[b]

[a] denotes significant difference to that recovered at 3 mins ($p<0.05$)

[b] denotes significant difference to that recovered at 3 mins and 3 h ($p<0.05$)

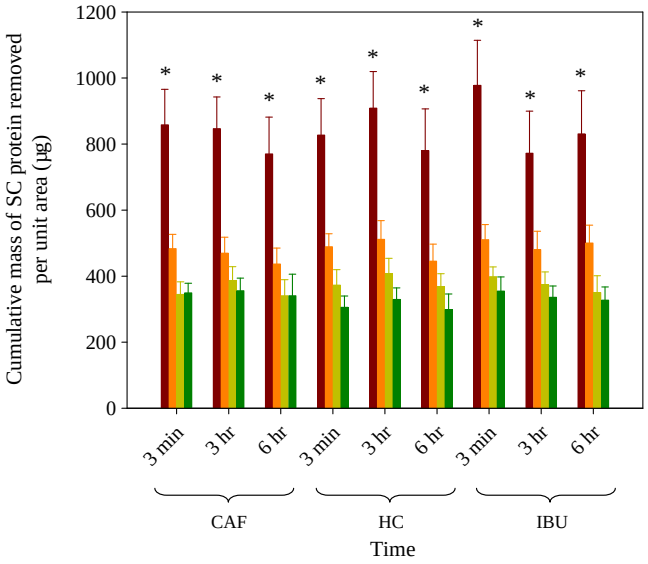

Fig. 5 Cumulative mass of protein removed per unit area from section 1 (■), section 2 (■), section 3 (■) and section 4 (■) following application of CAF, HC and IBU at 3 mins, 3 h and 6 h (mean ± SEM, $n=8$). * Indicates that the cumulative mass of protein removed from section 1 is significantly different ($p<0.05$) to the mass removed from sections 2, 3 and 4.

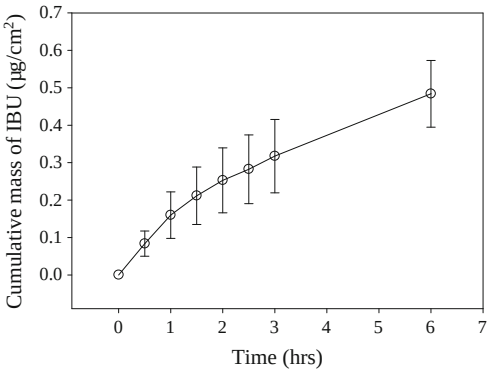

Fig. 6 *In vitro* permeation profile of IBU across dermatomed human skin (mean ± SEM, $n=8$).

concentric adhesive tape over time when ethanolic solutions of CAF, HC and IBU were applied.

It is evident that neither drug type nor length of exposure time affected the reproducibility of SC protein removal with adhesive tape. However, the cumulative mass of SC protein removed from skin sites directly under section 1 was significantly higher ($p<0.05$) than the cumulative amount removed at sections 1, 2 and 3, using a one-way ANOVA statistical test.

In Vitro Permeation Study

The permeability profile of IBU through dermatomed human skin is shown in Fig. 6. A total of 1.4±0.3 µg of IBU was detected in the receptor solution after 6 h, equating to 1.6±0.3 % of the applied dose, while no CAF or HC could be detected in the receptor fluid after 6 h, using the validated assay. This suggests that the decrease in IBU recovery from the uppermost regions of the SC is related to factors other than penetration through the skin, since only a small fraction of IBU appears to penetrate through the skin.

DISCUSSION

Tape stripping has commonly been used to follow the distribution profile of topically applied permeants across skin. However, the study of penetration as well as lateral diffusion of permeants on the surface of the SC and along the plane of the lipid bilayers within the SC using concentric adhesive tapes is not well documented. Furthermore, though it is widely understood that drug lipophilicity greatly impacts skin penetration (21,22), the impact on lipophilicity on lateral diffusion of drug has not yet been elucidated. This study demonstrated that the logP of permeants not only affects depth of penetration into the SC, but the distance which a drug spreads laterally from its site of application.

CAF, a hydrophilic drug with a logP of −0.07 (9) formed a flat reservoir extending into section 3 of the SC surface at 3 mins after dosing (Fig. 3a). Lateral diffusion on the surface of the SC and within the SC bilayers appears to have occurred quite rapidly as the concentration of CAF detected in section 2 is comparable to that detected in section 1. The majority of drug resided close to the surface of the skin (within 600 µg/area) and depleted with time. On the other hand, HC, a relatively more lipophilic drug with a logP of 1.43 (11) exhibited a lower tendency to spread laterally and instead, the majority of drug was contained within the first two sections at 3 mins after application. Upon closer inspection of Fig. 4c, it is revealed that the concentration of HC in section 2 is approximately half of that extracted in section 1 of the corresponding tape strips, thus giving rise to a deep and narrow drug depot on the skin surface and further demonstrating less lateral movement. The depot formation of HC is in agreement with studies performed by Malkinson *et al.* (23), whereby HC penetrated the skin surface but did not readily permeate out of the SC. In addition, HC may cause vasoconstriction of the local capillaries which in turn may slow down its removal rate from the SC and consequently instigate the formation of a drug reservoir or depot that releases drug over a period of several days (24,25).

HC also penetrated a greater depth of SC compared to CAF—having reached 800 µg/area after 3 mins of application. This is most likely due to the influence of lipophilicity on drug penetration (8). Conversely, the hydrophilic nature of CAF resulted in a wide and flat drug reservoir in the

uppermost layers of the SC and lower penetration. The higher spreadability of CAF on the SC surface may also be attributed to the formation of a layer of moisture on the skin surface (26). As a moisture layer is aqueous in nature, this may facilitate the lateral movement of a more water soluble compound such as CAF. HC, on the other hand, was concentrated within section 1 which covered a small contact area of 0.5 cm^2 and thus formed a deeper drug depot with drug penetrating further into the SC.

IBU has an ideal logP for skin penetration (a value of 3.51 (11)) and is observed to form both a wide and deep drug reservoir soon after dosing. IBU is detected to have spread into section 3 and reaches a SC depth of 1000 μg/area within 3 mins as seen in Figs. 3g and 4b. However, it should be acknowledged that, as approximately 1000 μg/area of SC protein is removed from the combined 10 tape strips (regardless of drug applied or duration of contact), it is plausible to assume that some IBU may have penetrated beyond the SC depth which was sampled. The SC may be pictured as being composed of protein bricks (keratin) in a continuous phase of lipophilic mortar, with paths through which hydrophilic and lipophilic substances can traverse (27). As lipophilic drugs are soluble in the continuous lipid mortar, diffusion through the SC poses less resistance as these drugs do so whilst staying in a favourable environment (28). In addition, it is accepted that the increase in drug lipophilicity may increase the ability of drugs to disrupt the lipophilic domains of the SC and therefore facilitate the diffusion of drugs across the SC (11,29). Thus, IBU is observed to penetrate deeper into the SC compared to CAF and HC.

The rapid rate of percutaneous penetration and lateral redistribution of lipophilic substances in *in vivo* animal models was also demonstrated by Simonsen *et al.* (30). By collecting biopsies at distances of 1.3 and 2.9 cm from the application area, it was shown that a much higher concentration of butyl salicylate (logP of 4.63) was found at various distances from the application area in comparison to those of salicylic acid (logP 2.26). The higher degree of lateral distribution of butyl salicylate was explained by its lipophilicity which favoured partitioning into the SC and most probably lateral diffusion. However, it is unknown whether lateral diffusion occurred on the SC surface or within the SC bilayers as drug was extracted from skin biopsies with the entire SC intact.

It has been suggested that lateral diffusion is strongly dependent on molecular size and pronounced for compounds with a molecular size up to 300 g/mol (30). This is in good agreement with our results as CAF and IBU, which have a molecular size of 194.46 and 206.28 g/mol, respectively, exhibited greater lateral diffusion than HC (with a molecular size of 362.46 g/mol). Therefore, the lateral diffusion of topically-applied drugs may be dependent on the drug's solubility within the moisture layer on the skin surface, its lipophilicity, as well as its molecular weight. In addition, as the small dose of ethanol evaporated soon after application, CAF, HC and IBU may exist at a saturated concentration or precipitate onto the skin surface or within the skin depending on their solubility state. It is therefore possible that the spreading and/or penetration behaviours of the model drugs are affected by this vehicle effect. A common trend observed between the distribution of CAF, HC and IBU from the above studies is that the concentration of drug was highest at the surface and then decreased with depth of SC and radial distance from the application site (Fig. 3). There was no significant difference in the distribution profile of permeants at sites directly below the application area over 6 h. This may be due to a higher drug loading at the initial site of contact which presents a steeper concentration gradient across the SC (at the application site) that provides the main driving force for the diffusion through skin.

Previous studies carried out by Rhodes *et al.* (31) suggest that only 50 % of the applied formulation remains on the skin surface 8 h after application, with observed differences between formulation types (32). This is in agreement with the results in the present study listed in Table III where between 47 and 65 % of CAF, HC and IBU was recovered from the skin at 6 h after application. The reduced recovery of CAF and HC from the outermost regions of the SC at 3 h and 6 h is mainly due to depletion of drug from section 2 and 3 of the sampling sites (as seen in Fig. 3), as no increase in CAF and HC penetration into the deeper skin regions or lateral diffusion was revealed with an increase in time. In contrast, the reduction of IBU detected in section 2 of the sampling site is reflected by an increase in lateral diffusion and/or penetration (from preceding SC region) into section 3 in the deeper regions of the sampled SC at 6 h as demonstrated in Fig. 3i.

The deeper penetration of IBU through human skin, relative to CAF and HC, is further highlighted by the *in vitro* permeation of IBU as shown in Fig. 6. Following a 6 h period, 1.6 ± 0.3 % of the applied dose of IBU was detected in the receptor fluid, whereas no detectable amounts of CAF or HC were present. Unlike the documented *in vitro* permeation studies where the concentration of CAF or HC dosed was at saturated concentrations (16,33–37) or in the presence of additives that influence penetration (38,39), the lower drug loading in addition to the sub-saturated dosing concentration is most likely responsible for the absence of CAF and HC in the receptor fluid. However, some studies have found that even at saturated concentrations, a longer lag time of >6 h is required before any CAF (33,34) or HC (39) is detected in the receptor fluid. Even more interestingly, only approximately 1 % of HC is detected after 5 days (40) and 10 days (41) following application. It is therefore,

no surprise, to observe that CAF and HC exhibit limited penetration across the SC, in line with our *in vivo* studies.

As only IBU was detected in the receptor fluid at 6 h after application *in vitro*, it is reasonable to assume that the loss of IBU *in vivo* from the SC surface at the equivalent time point was only partially due to absorption into the deeper viable skin tissue. However, as the percutaneous absorption of IBU was approximately 1.6 % of the applied dose, the majority of the *in vivo* loss of IBU, CAF and HC from the SC surface at 6 h after exposure may largely be due to depletion via skin shedding, metabolism or simply by being rubbed off, thus being lost to the surrounding environment.

In addition to following the distribution profile of permeants across and within the SC, this study also allowed us to determine whether the choice of drug and duration of drug-to-skin contact had an effect on the amount of SC protein removed via tape stripping as a function of distance from the application site. As shown in Fig. 5, neither drug type nor length of skin contact influenced SC protein removal. However, it appears that the cumulative mass of SC protein removed from sites directly under the application area (section 1) was significantly higher than the cumulative mass of protein removed at distances further from the application area. This suggests that the amount of drug loading at a particular site may be indicative of the amount of protein removed as the results above show that a higher drug content is found in section 1 and progressively decreases with distance. However, it should be acknowledged that although all efforts were made to ensure even pressure was applied to tape strips so as to remove a uniform weight of SC protein across the tape, it is still possible that (following splitting of the concentric tapes in half) that one half of the concentric tape removed more SC protein than the other due to the presence of furrows (42). Therefore, the cumulative mass of SC protein reported in this research may over estimate or under estimate the total mass of SC protein removed.

CONCLUSION

Using a novel concentric ring tape-stripping technique, it was demonstrated that the lateral spreading behaviour of drugs appears to be dependent on the physicochemical properties of the drugs applied. CAF and IBU demonstrated a higher tendency to undergo lateral diffusion while HC formed a drug depot at the application site. Furthermore, the extent of penetration appears to be dependent on physicochemical properties with IBU exhibiting greater penetration and with CAF and HC remaining in the uppermost regions of the SC. In addition, the greater loss of IBU from the SC *in vivo* appears to be related to its greater penetration through the SC as observed *in vitro*.

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