Topical penetration of commercial salicylate esters and salts using human isolated skin and clinical microdialysis studies

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Aims The penetration of active ingredients from topically applied anti-inflammatory pharmaceutical products into tissues below the skin is the basis of their therapeutic efficacy. There is still controversy as to whether these agents are capable of direct penetration by diffusion through the tissues or whether redistribution in the systemic circulation is responsible for their tissue deposition below the application site.

Methods The extent of direct penetration of salicylate from commercial ester and salt formulations into the dermal and subcutaneous tissue of human volunteers was determined using the technique of cutaneous microdialysis. We also examined differences in the extent of hydrolysis of the methylester of salicylate applied topically in human volunteers and in vitro skin diffusion cells using full-thickness skin and epidermal membranes.

Results The present study showed that whilst significant levels of salicylate could be detected in the dermis and subcutaneous tissue of volunteers treated with the methylsalicylate formulation, negligible levels of salicylate were seen following application of the triethanolamine salicylate formulation. The tissue levels of salicylate from the methylsalicylate formulation were approx. 30-fold higher than the plasma concentrations.

Conclusion The absorption and tissue concentration profiles for the commercial methylsalicylate formulation are indicative of direct tissue penetration and not solely redistribution by the systemic blood supply.

Keywords: methylsalicylate, glycolsalicylate, triethanolamine, percutaneous

Introduction

Despite its evolved barrier properties, the skin is an important route of entry into the body for many topically applied solutes. Topical pharmaceutical products targeted locally below the site of skin application are assumed to exert their effect by penetration of the active ingredient directly into deeper tissues, though there is still controversy over whether many agents are in fact removed by the dermal blood supply before they reach their desired site of action. In a study by Dawson et al. [1] synovial fluid concentrations of the antiinflammatory agent biphenylacetic acid (felbinac) in arthritic patients following application of 3g of a 3% gel were found to be similar in treated and control knees and were only approximately half that seen in plasma, suggesting that no direct penetration of the drug into the treated knee joint had occurred. More traditional methods for the assessment of efficacy of topical products in human volunteers rely predominantly on kinetic analysis of plasma, urinary excretion and faecal drug and/or metabolite profiles [2]. Other techniques such as analysis of drug remaining on the skin, determination of pharmacodynamic end points, tissue biopsy, suction blister fluid analysis and skin windows have also been used though the latter direct assessments of tissue concentrations are limited in use because of their tissue-destructive nature, their inability to give multiple time samples from the same site and their likelihood of resultant temporary or permanent scarring. Despite its evolved barrier properties, the skin is an important route of entry into the body for many topically applied solutes. Topical pharmaceutical products targeted locally below the site of skin application are assumed to exert their effect by penetration of the active ingredient directly into deeper tissues, though there is still controversy over whether many agents are in fact removed by the dermal blood supply before they reach their desired site of action. In a study by Dawson et al. [1] synovial fluid concentrations of the antiinflammatory agent biphenylacetic acid (felbinac) in arthritic patients following application of 3g of a 3% gel were found to be similar in treated and control knees and were only approximately half that seen in plasma, suggesting that no direct penetration of the drug into the treated knee joint had occurred. More traditional methods for the assessment of efficacy of topical products in human volunteers rely predominantly on kinetic analysis of plasma, urinary excretion and faecal drug and/or metabolite profiles [2]. Other techniques such as analysis of drug remaining on the skin, determination of pharmacodynamic end points, tissue biopsy, suction blister fluid analysis and skin windows have also been used though the latter direct assessments of tissue concentrations are limited in use because of their tissue-destructive nature, their inability to give multiple time samples from the same site and their likelihood of resultant temporary or permanent scarring.

One of the most popular over-the-counter groups of topical agents are the salicylate-containing anti-inflammatory formulations (most commonly methylsalicylate (MeSA) and triethanolamine salicylate (TEASA)) recommended for use in a wide range of painful or inflammatory conditions in muscle and joints and as passive pre-sports muscle warm ups. It has been shown that first-pass metabolism exists in the skin with esterases rapidly hydrolysing salicylate esters to release the active salicylate in both the epidermis and dermis [3]. A number of classical bioavailability studies in humans have examined the kinetic profiles of topical salicylate formulations and determined the effects of various factors such as site of application, multiple dosing and heat and exercise based on analysis of plasma levels and urinary excretion. Roberts et al. [4] found that the urinary excretion of salicylate and metabolites from a MeSA formulation in humans was site dependent, highest when applied to the abdomen rather than identical surface areas on the forearm or lower limb. A more recent study by Morra et al. [5], showed that the absorption rate of topical MeSA also increased with repeated application and that salicylate bioavailability was significantly higher from this formulation than from a topical formulation containing TEASA. Exercise
and heat exposure has been shown to increase the plasma concentrations and urinary excretion of topical MeSA by up to three times, highlighting the importance of blood flow on absorption kinetics from topical sites [6]. None of the above studies, however, addressed the local tissue concentrations of active ingredients following applications of the topical formulations, the presence or absence of which forms the basis of their therapeutic efficacy. In the present study we used cutaneous microdialysis to determine the direct local penetration of topically applied nonsteroidal anti-inflammatory formulations containing esters or salts of salicylate.

Cutaneous microdialysis is a relatively new technique which allows the continuous monitoring of endogenous or exogenous solutes in the interstitial fluid of dermal or subcutaneous tissue with minimal tissue destruction. The technique involves the placement of small perfused probes into the skin at given depths (e.g. dermis or subcutaneous tissue). The ends of these probes incorporate a small dialysis membrane window across which solutes can exchange from the interstitial fluid. Since the first report of microdialysis sampling in dermis [7], the technique has been used successfully in human volunteers to study the endogenous cutaneous release of histamine in response to various topical stimuli [8] and the penetration of a number of topically applied organic solvents (e.g. ethanol, isopropanol) [9]. Microdialysis is also gaining use in the characterisation of transdermal transport [10], to date examining drugs such as nicotine and lignocaine [11, 12] through the skin. In the present study, we hypothesised that based on our previous animal data [13–15], there would be selective deep tissue penetration of active salicylate from esters and salts present in commercial formulations. We have published some preliminary evidence that direct deep tissue penetration can occur in man [16]. It was anticipated that comparison of in vivo human skin diffusion profiles and in vivo human microdialysis data with plasma levels would enable the extent of direct deep tissue penetration of salicylates to be quantified in humans. We further wished to determine the extent to which such penetration is achieved with commercial salt and ester formulations.

Methods

In vitro diffusion

Human breast skin from plastic surgical procedures was collected following surgery and stored frozen (−20 °C) until use. Thawed and hydrated full-thickness skin was cleared of any excess subcutaneous tissue, cut into approximately 15 × 15 mm pieces and mounted, stratum corneum uppermost, in Franz-type glass diffusion cells, surface area 1.3cm². Human epidermal membranes were also prepared from breast skin using the heat separation method and mounted in the diffusion cells over fine cotton gauze to provide support. Skin samples (full-thickness or epidermal membranes) were allowed to equilibrate for 1 h in a water bath at 35 °C over receptor fluid (degassed, 20% ethanol; 80% distilled water) continuously stirred with magnetic flasks. At time zero 1g of topical commercial formulation containing 20% MeSA, 7% glycol salicylate (GSA) or 10% TEASA (Figure 1) was placed onto the stratum corneum side of the skin and receptor fluid removed and replaced with fresh solution at 1, 2, 3, 4, 5, 6, 8, 22 and 24 h. Due to the limited number of skin samples harvested from the donor, and to avoid complication of comparison across human samples, only full-thickness in vitro membranes were studied for the GSA formulation. Samples were assayed for parent salicylate ester and salicylate by h.p.l.c. At the end of each study, diffusion cells were dismantled, skin samples cleared of remaining formulation and wiped with alcohol swabs, the stratum corneum side stripped once with Scotch Tape™ and sample area exposed to formulation excised and placed into preweighed vials. Epidermis or 100 mg of finely chopped full-thickness skin were minced with scissors in 380 µl acetonitrile to which 100 µl of distilled water and 20 µl of 35% phosphoric acid was added. Samples were then vortexed, sonicated on ice for 30 s, centrifuged and the supernatant injected onto the h.p.l.c.

In vivo microdialysis

Human in vivo microdialysis was performed in consenting volunteers and approved by both the University of Queensland and Princess Alexandra Hospital Research Ethics Committees. Microdialysis probes with a MW cut off of 20,000 Da (CMA 70, CMA Microdialysis Roslagsvägen 101−, Stockholm) were inserted onto a guide (16G 57 mm Jelco i.v. placement unit) into the dermis or subcutaneous tissue through a 3 mm intradermal weal of lignocaine (10 mg ml⁻¹) on swabbed (alcohol or chlorhexidine solution) ventral forearm skin of volunteers (mean ± s.e.mean, 29.4 ± 1.9 years). A total of 17 probes (maximum 2 probes per volunteer per study) were placed in seven people with at least 3 days washout and a separate arm used in repeat volunteers. A ‘dermal’ probe was inserted superficially so as to cause an axon reflex flare whilst a ‘subcutaneous’ probe was inserted superficially but not so as to produce a flare. Probes were taped in place, additionally secured with Opsite® semi-permeable transparent dressing and perfused continuously with physiological saline at 380 µl h⁻¹.

![Figure 1 Structures of the active anti-inflammatory ingredients in the three salicylate formulations studied.](image-url)
Skin penetration of topical salicylates


with normal saline at 1.6 μl min⁻¹ using a portable syringe driver (MS16A, Graseby Medical, Gold Coast, Australia). Following 1 h probe equilibration, commercial topical salicylate formulations (as used in vitro) containing 20% MeSA, 7% GSA or 10% TEASA (Figure 1) were applied to a 16 cm² area of skin over the probe tips but at least 10 mm from covered probe insertion points. Dialysate samples were collected at 30–60 min intervals for up to 360 min. In two subjects application of TEASA was started 24 h before probe insertion. Subjects applied a liberal amount of formulation to the test area at 2–3 h intervals, except during the night, in order to achieve maximal concentrations at the test site. At the end of the sampling period, blood samples were taken from the vein in the treated arm in each volunteer. Blood was centrifuged, plasma protein precipitated by 0.05 M acetonitrile at pH 3, flow rates used were 1.0 and 1.2 ml min⁻¹ for in vitro analysis of salicylate and salicylate esters in samples from estimated to be four orders of magnitude greater than from known concentrations in 2% bovine serum albumin buffer at pH 7.4. Recovery was used to adjust dialyse concentrations to those estimated to be present just outside the probe tip dialysis membrane.

Sample analysis

High performance liquid chromatography was used for the analysis of salicylate and salicylate esters in samples from both in vitro and in vivo studies. Mobile phase consisted of 50:50 acetonitrile:0.05 M potassium phosphate buffer at pH 3, flow rates used were 1.0 and 1.2 ml min⁻¹, injection volume was 30 μl (in vitro study) and 10 μl (in vivo study) onto a χ-Bondapack C18 (3.9 × 300 mm) column (Waters, Millipore, Australia) with detection by either u.v. (salicylate and salicylate ester)(s) at 237 nm or fluorescence (in vivo salicylate)(Excitation λ 295 nm, emission λ 400 nm) over a 15 min run time. Two Shimadzu hardware systems (Shimadzu Oceania Pty Ltd, Australia) were used consisting of (i) a LC-6AD pump, SIL-6B autoinjector and a SPD-6A UV detector and (ii) a RF-10AXL fluorescence detector.

Data analysis

Slopes from linear regression of plots of receptor fluid concentration vs time from in vitro diffusion cells were used to calculate the permeability as flux of salicylate and salicylate esters through human skin.

Results

In vitro

Substantial amounts of salicylate and its parent esters penetrated human skin in vitro. The permeability as flux calculated from the cumulative amount vs time plots together with the relative amount of total salicylate or parent ester penetrating full-thickness skin or epidermal membranes in 24 h from each formulation are shown in Table 1. Salicylic acid was freely soluble in the receptor phase and MeSA was soluble to over 500 μg ml⁻¹, which was well in excess of drug concentrations determined throughout the in vitro studies, suggesting that sink conditions were adequate. Permeability as flux of active ingredients from the methyl ester and TEA salt formulations of salicylate through human epidermal membranes were approximately three-fold faster than through full-thickness skin. The ratio of MeSA to salicylate from the MeSA formulation appeared to remain relatively constant at around 3:1 for both full-thickness skin and epidermal membranes. In full-thickness skin studies no free salicylate was detected in the receptor phase of cells treated with the GSA formulation.

Comparison of the amount of drug remaining in full-thickness skin and epidermal membrane samples at the end of each of the studies following application of MeSA and TEASA formulations (Table 1) showed that the epidermis appeared to be the major site for deposition of salicylate from the salt and to some extent that generated from the methylester salicylate formulation. Dermal deposits of salicylates were determined by subtraction of the amount of drug determined in the epidermal samples from that found in full-thickness skin, and accounting for equivalent fraction of epidermis weight (0.0103 ± 0.004 g cell) in the full-thickness samples (0.2808 ± 0.096 g cell). It was estimated that over 99.9% of the salicylate present in full-thickness skin samples treated with the TEASA formulation could be accounted for in the epidermis. The penetration of the methylester into the dermis of full-thickness skin samples in vitro was estimated to be four orders of magnitude greater than from the salt formulation.

In vivo

Recovery of salicylate into probes was 30.7 ± 3.5%, mean ± s.e.mean, with all reported dialyse levels adjusted by this amount to allow estimation of corresponding tissue levels immediately outside the membrane. Significant amounts of salicylate were found in the dialyse from both dermal and subcutaneous probes in volunteers receiving the methylsalicylate and glycolsalicylate formulations, but not the TEASA formulation (Figure 2). The cumulative amount of salicylate present in the dermis estimated from dialyse following approximately 5 h of application was over 80-fold higher in volunteers treated with the 20% MeSA compared with the 10% TEASA formulation. Salicylate dialyse to plasma ratios (Table 2) showed that estimated tissue concentrations were substantially higher than that in the circulating blood following application of salicylate ester formulations but not the TEASA salt.

The time course of salicylate penetration into dialyse was fairly rapid for the ester formulations. Salicylate from the MeSA formulation could be detected within 1 h of application and continued to rise rapidly over the first 30–90 min then appeared to plateau, with reaplication of the dose at 2 h showing a repeat in this pattern of increase (Figure 2a). In the two volunteers receiving glycolsalicylate, dermal levels were apparent at 60–90 min following application and appeared to plateau after 2 h at around 1.72 μg ml⁻¹, even following reaplication of the formu-
lation, which corresponded to the steady state levels seen in a single volunteer predosed with the formulation for 24 h previous to the study (Figure 2b). The levels of salicylate in both dermal and subcutaneous probes were low and near the limit of detection of the fluorescence assay (50 ng ml$^{-1}$) following application of the TEASA formulation in all four volunteers, including the two volunteers predosed for 24 h with the formulation.

In all of the analyses of in vivo samples only salicylate could be detected in the dialysate and plasma of volunteers, with no evidence of either the parent MeSA or GSA ester applied in the formulation. In order to increase the limit of detection of the h.p.l.c. assay to 10 ng ml$^{-1}$, dialysate samples from volunteers were pooled for each individual and five times the volume injected, however there were still no detectable parent salicylate ester peaks.

Discussion

The human skin permeability as flux generated by the in vivo studies showed that substantial amounts of salicylate penetrated skin by diffusion alone from all of the formulations tested, particularly the GSA gel. The significant amounts of parent salicylate ester penetrating into the receptor fluid from MeSA and GSA formulations suggested a relatively low rate of hydrolysis of these solutes by skin esterases. However, the human breast skin used had been stored frozen at $-20^\circ$C prior to the study and therefore the activity of esterases within the tissue would not have been equivalent to that seen in vivo. Due to high esterase activity in living skin [3], the levels of salicylate esters determined in receptor fluid could be assumed to exist primarily as salicylate when extrapolating the observed permeabilities to the in vivo situation. In vivo human microdialysis confirmed the high esterase activity in viable skin as no trace of parent ester from the MeSA and GSA formulations were observed, though their ability to diffuse as the parent moiety had been proven in vivo. Figure 3 summarises the difference in levels of salicylate generated from MeSA observed in the present in vivo and in vitro studies. Boehnlein et al. [17] have shown similar differences in esterase activity between viable and non-viable skin without any corresponding change in the percutaneous absorption rates of MeSA or benzyl alcohol in guinea pig skin. The group also suggested that the rate of skin permeation of MeSA was determined by diffusion through the stratum corneum and that the primary metabolite seems to occur in the viable tissues beneath the stratum corneum after the rate of permeation has been determined.

The epidermal membranes produced, as expected, higher permeability as flux than the full-thickness skin samples in vivo. However, the ratio of fluxes of the salicylates absorbed into the receptor phase from the methyl ester compared with the TEASA salt formulation remained relatively constant across the two membranes, a ratio of 1.6 for epidermal membranes and 1.8 for full-thickness skin samples. These data suggest that the dermis constitutes a significant barrier to the diffusion of active ingredients from both formulations as, other than the extra time taken to diffuse across this unstirred layer in vivo, no significant changes to the absorption profiles from either formulation were observed. This finding also suggests that the TEASA formulation has approximately twice the bioequivalency of the MeSA cream. The TEASA cream contained around one quarter the amount of salicylate by weight as the MeSA formulation, as the TEA accounts for half of its weight, and an equivalent ratio active ingredient flux of 4 would have been predicted. The in vivo microdialysis data, however, suggested a lack of correlation between the permeability of salicylate from the TEASA formulation based on the in vitro findings. The in vivo permeability as flux for the active salicylate in the TEA salt formulation was approximately 60% of that observed for the combination of MeSA and salicylate from the MeSA formulation. Based on this finding, it could be assumed that levels of salicylate following in vivo application of the TEASA formulation would be

<table>
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<tr>
<th>Formulation Applied</th>
<th>Flux (mg cm$^{-2}$ h$^{-1}$)</th>
<th>$s.e.$</th>
<th>Min.</th>
<th>Max.</th>
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<tr>
<td>20% MeSA</td>
<td>F-T</td>
<td>11.2±0.7</td>
<td>32.8±2.0</td>
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<tr>
<td></td>
<td>Epi</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60% TEASA</td>
<td>F-T</td>
<td>3.8±0.2</td>
<td>13.1±1.0</td>
<td>8.7±0.7</td>
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<tr>
<td></td>
<td>Epi</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>7% GlySA</td>
<td>F-T</td>
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<tr>
<td></td>
<td>Epi</td>
<td>-</td>
<td>-</td>
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Table 1  In vitro permeability as flux and amount remaining in skin samples of salicylate and salicylate esters applied to full-thickness skin (F-T) or epidermal membranes (Epi) from human breast following application of topical salicylate formulations. Me e+s.e.mean, min. n=8.

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The *in vitro* finding of high deposition of salicylate from the TEASA formulation in the epidermis compared with the MeSA product may have some bearing on the lack of correlation between the *in vitro* and *in vivo* results. It is possible that in *vivo* salicylate in the epidermis is not being released as quickly as *in vitro* due to differences in sink conditions or stronger binding to viable or other tissue constituents. These effects would result in a low and prolonged release of salicylate into the systemic circulation or tissue surrounding the microdialysis probe at concentrations near or below the level of detection of the assays employed in the present study. The ratio of total active ingredients (MeSA plus salicylate) estimated to be present in the dermis from the TEASA compared with MeSA formulation were extremely low in both studies, with values of 0.003 and 0.032 *in vitro* and *in vivo* respectively. The combination of this finding with the observed *in vitro* permeability as flux suggests that whilst salicylate from the TEASA formulation is strongly associated with the epidermis, its release and diffusion across the dermis *in vitro* must be fairly rapid, resulting in a reasonably high accumulation in the receptor and subsequent calculated flux. A differential penetration of salicylic acid and salicylate salts into multilayer membrane systems and the human horn layer *in vitro* was noted by Neubert et al. [18]. This group found that whilst salicylic acid penetrated rapidly into all membranes the sodium and choline salts of salicylate accumulated in the first membrane. Pardo et al. [19] suggested that, following application of a physostigmine ion-pair of salicylate, ionization of ion-pairs at the pH of hydrated stratum corneum occurred immediately after partitioning into the membrane and subsequent diffusion of the ionized species across the membrane. In the present study it could also be anticipated that the TEASA ion-pair would dissociate upon partitioning into the membrane resulting in liberation of ionized salicylate which would have much slower diffusion and higher binding in the lipophilic epidermal regions than through the relatively aqueous environment of the dermis.

In animal studies, Singh & Roberts [14] showed that the importance of systemic blood levels in accounting for salicylate concentrations in underlying tissues after dermal application was related to (i) the depth of the tissue, (ii) the time of application, and (iii) the tissue blood flow. Deep tissue penetration by direct diffusion or other means was likely to be most evident in the more superficial tissues immediately after topical application. The tissue concentrations tend to fall exponentially with depth due to the tissue clearance of salicylate by perfusing blood in each tissue the salicylate may penetrate into. The time dependency of systemic blood determined tissue concentrations depends on the accumulation of sufficient salicylate into the systemic circulation from the blood perfusing each tissue and the relative penetration rate of salicylate into deeper tissues [14]. The importance of the third determinant of tissue blood flow is evident in the perfused limb work of Cross & Roberts [20], vasconstriction work of Singh & Roberts [21] and in the tissue depth-salicylate concentration relationships in alive and sacrificed animals [15]. This work shows that an increase in dermal and other tissue clearance as a consequence of an increase in tissue blood flow is reflected by a decrease in underlying tissue levels below an application.
Table 2 Human in vivo steady-state salicylate estimated tissue concentrations and tissue to plasma ratios following application of topical formulations.

<table>
<thead>
<tr>
<th></th>
<th>Volunteer 1</th>
<th>Volunteer 2</th>
<th>Volunteer 3</th>
<th>Volunteer 4</th>
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<tr>
<td><strong>Dermal 20% Methylsalicylate</strong></td>
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<td>Tissue concentration (µg mL⁻¹)</td>
<td>2.8</td>
<td>2.4</td>
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<tr>
<td>Tissue/Plasma ratio</td>
<td>44.1</td>
<td>27.8</td>
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<tr>
<td><strong>Subcutaneous</strong></td>
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<tr>
<td>Tissue concentration (µg mL⁻¹)</td>
<td>2.7</td>
<td>4.1</td>
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<tr>
<td>Tissue/Plasma ratio</td>
<td>42.9</td>
<td>46.6</td>
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<td><strong>Dermal 10% Triethanolamine salicylate</strong></td>
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<tr>
<td>Tissue concentration (µg mL⁻¹)</td>
<td>0.04*</td>
<td>0.001</td>
<td>0.02</td>
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<tr>
<td>Tissue/Plasma ratio</td>
<td>1.8*</td>
<td>0.1</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Subcutaneous</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tissue concentration (µg mL⁻¹)</td>
<td>0.03*</td>
<td>0.001</td>
<td>0.003</td>
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<tr>
<td>Tissue/Plasma ratio</td>
<td>1.5*</td>
<td>0.1</td>
<td>0.6</td>
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<tbody>
<tr>
<td><strong>Dermal 7% Glycolsalicylate</strong></td>
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<tr>
<td>Tissue concentration (µg mL⁻¹)</td>
<td>2.9</td>
<td>0.5</td>
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<tr>
<td>Tissue/Plasma ratio</td>
<td>5.6</td>
<td>2.4</td>
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<sup>*</sup>Concentrations at limit of assay detection.

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Figure 3 Fraction of total concentration of active ingredients determined in microdialysate or in vitro diffusion cell studies to be present as salicylate following topical application of the 20% MeSA formulations. Mean ± s.e.mean, n = min 3.

The present study clearly shows, however, that direct local tissue penetration of active ingredient was achieved with the MeSA and GSA formulations. The high tissue/plasma ratios observed demonstrate that distribution of the solute in the plasma was not responsible for the tissue levels determined by microdialysis. If redistribution of the solutes into deeper tissues following clearance by the dermal blood supply was occurring then tissue/plasma ratios would have been expected to be unity or below, as observed in the case of the TEASA formulation which showed no evidence of direct tissue penetration following topical application.

The study also highlights the importance of the technique of microdialysis in enabling continuous monitoring of tissue levels of topically applied solutes for the determination of their in vivo pharmacokinetics. Pharmacokinetic studies relying on plasma concentration-time profiles and urinary excretion data provide little information as to the local distribution of solutes in tissues directly below a topical application site. In the case of the non-steroidal anti-
inflammatory formulations examined in the present study, their assumed ability to penetrate into deeper tissues underlying their application site is used as the basis for their clinical efficacy. It should therefore be remembered that in vivo human skin permeability data may not necessarily be used as an indication of in vivo efficacy for many topical formulations. The introduction of the technique of in vivo human cutaneous microdialysis or other methods of determining local tissue levels of solutes following topical application in humans may become ultimately more important in proving the human in vivo efficacy of formulations requiring direct penetration to tissues below a topical application site.

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