Self Promotion of Deep Tissue Penetration and Distribution of Methylsalicylate After Topical Application

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Purpose. To determine how changes in cutaneous blood flow induced in-vivo by methylsalicylate (MeSA), compared to non-rubefacient triethanolamine salicylate (TSA), affected topical salicylate absorption and distribution, and to assess formulation therapeutic potential by comparing tissue concentrations to published antiinflammatory concentrations.

Methods. Flux of salicylate from MeSA and TSA formulations applied to full-thickness rat skin was determined using in vitro diffusion cells. Anaesthetised rats were then used to quantify salicylate concentrations in plasma and tissues underlying the application site for the two formulations over a 6h period. In vitro and in vivo absorption profiles were then compared and the effect of MeSA on cutaneous blood flow assessed.

Results. In vitro flux of salicylate from the MeSA formulation was 40% higher, though after correcting for differences in formulation concentrations the ratio of permeability coefficients was reversed. Contrary to the *in vitro* predictions, *in vivo* tissue and plasma concentrations of salicylate in rats rose rapidly in the first 1 hr and were more than the predicted 1.4-fold higher for MeSA. This effect was mirrored by the increase in blood flow induced by MeSA in human cutaneous vessels and that reported in the literature. Potential therapeutic levels were not seen below superficial muscle layers.

Conclusions. Direct tissue penetration of salicylate occurs below application sites from both MeSA and TSA formulations. Tissue concentrations of MeSA were higher than predicted due to its rapid distribution in the blood.

KEY WORDS: rats; percutaneous; salicylates; metabolism.

INTRODUCTION

Topically applied salicylate anti-inflammatory and pain relieving rubs are rapidly becoming one of the most popular over-the-counter treatments for muscle aches and temporary relief of joint pain. A number of clinical reports have examined the efficacy of topical versus oral salicylate therapy in the relief of arthritic pain and found contradicting results. An early study by Golden (1) in a double-blind comparison between oral aspirin and topical triethanolamine salicylate (TSA) showed that the topical cream was at least as effective as 650 mg of oral aspirin and provided quicker relief with fewer side effects. However, Algozzine *et al.* (2) argued that this early study had not used patients as their own controls and so they performed another trial testing 10% TSA cream against a placebo control in a crossover study. The group found no significant difference between the ability of TSA and placebo creams to relieve symptomatic osteoarthritis of the knee in 25 patients, with 6 patients preferring the placebo and 11 having no preference.

It is assumed that anti-inflammatory ingredients in topical formulations penetrate the skin and reach deeper tissue layers below the subcutaneous tissue to provide therapeutic effects. Most studies evaluating the topical penetration of salicylate esters and salts are based on plasma profiles and urinary excretion data (3,4). We recently used cutaneous microdialysis to show that dermal and subcutaneous tissue levels of salicylate in man are significantly higher after application of a methylsalicylate (MeSA) formulation than for a TSA product (5). Tissue levels were estimated to be 30-fold higher than the circulating plasma concentrations, suggested to play a dominant role in distribution of some topical anti-inflammatory drugs into underlying tissues (6,7). Animal studies have been used to demonstrate effective deep tissue penetration from topical sites (8,9). Singh and Roberts (9) examined the pharmacokinetics of salicylic acid distribution into underlying tissues following dermal application in rats, and suggested that direct penetration only occurred during the first 2 h of application to a depth of 3–4 mm (equivalent to dermis and subcutaneous tissue). At longer times, systemic recirculation of salicylate through the tissues dominated the observed tissue concentrations. It is thus apparent that the results of Radermacher *et al.* (7) and Dawson *et al.* (6) can be interpreted in terms of this model if it is recognised that their sampling times coincided with the recirculation phase suggested by Singh & Roberts (9).

In this paper, we compared the topical absorption of salicylate in its rubefacient form, MeSA, and as its non-irritating salt, TSA, (Fig. 1) following topical application in vitro and in vivo to rats. We were particularly interested in whether increases in cutaneous blood flow, induced by application of MeSA, affected salicylate tissue distribution since our previous in situ work with an artificially perfused rat hindlimb model has suggested that deep tissue penetration after dermal application of solutes is altered by increased vascular flow (10). We further attempted to gain an insight into the therapeutic potential of the two commercial salicylate formulations in muscle tissues underlying the application site by relating observed salicylate concentrations to published minimum anti-inflammatory concentrations.

METHODS

All animal experimentation was approved by the University of Queensland Animal Experimentation Ethics Committee and adhered to strict guidelines as defined by the National Health and Medical Research Council of Australia.

In Vitro Skin Penetration

Male Wistar rat full-thickness skin (from euthanased animals) was clipped, depilated with Nair® hair removal cream, cleared of any excess subcutaneous tissue, cut into approx. 15 × 15 mm pieces and mounted, stratum corneum uppermost, in Franz-type glass diffusion cells, surface area 1.3 cm^2. Skin samples were allowed to equilibrate for 1 h in a water bath at

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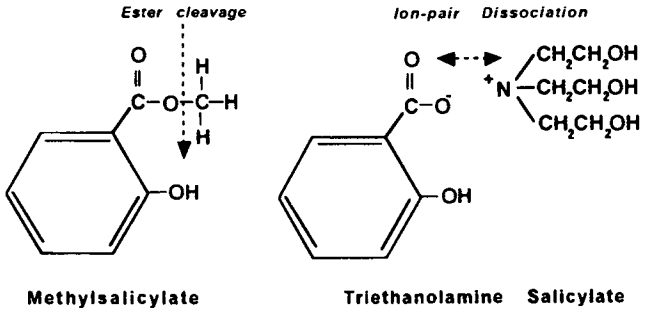

Fig. 1. Structure of (A) the triethanolamine ion-pair of salicylate which dissociates to yield free salicylate and (B) the methyl ester of salicylate which is hydrolysed by skin and other esterases to yield free salicylate.

35°C over receptor fluid (degassed, 20% ethanol:80% distilled water) continuously stirred with magnetic fleas. At time zero 1 g of formulation containing 10% TSA (Dencorub Arthritis Cream, Carter Wallace Aust. Pty Ltd, NSW) or 20% MeSA (Dencorub Pain Relieving Cream, Carter Wallace Aust. Pty Ltd, NSW) was placed onto skin and receptor fluid removed and replaced with fresh solution at various time points over the next 28 h. Samples were assayed for MeSA and salicylate by HPLC. The stability of TSA and MeSA in receptor fluid over a 4 day test period were 100 and 98.7% respectively. 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 cellotape and the sample area exposed to the formulation excised and placed into preweighed vials. 100 mg of skin was minced with scissors in 380 μl acetonitrile to which 100 μl of distilled water and 20 μl of 35% phosphoric acid were added. Samples were then vortexed, sonicated on ice for 30 s, centrifuged and the supernatant injected onto the HPLC.

In Vivo Absorption

1 g of each formulation was applied to a 9.6 cm^2 marked area of depilated abdominal skin of anaesthetised (phenobarbitone sodium, 60 mg kg^{-1}, i.p.) male Wistar rats (310 ± 20 g, n = 3 per time point). A blood sample was removed from the tail vein at 0.5, 1, 2, 4 and 6 h after which the rats were sacrificed by cervical dislocation whilst still under anaesthesia. Formulations were then removed from the skin with a spatula and the area wiped clean. Tissue samples (skin, subcutaneous tissue, superficial muscle, deep muscle and fat) from below the site of application and from an untreated contralateral site were sequentially excised. The epidermis was separated from the dermis by exposure of the excised skin to ammonia fumes for 1 h followed by removal with a surgical blade. The epidermis was discarded. Blood samples were centrifuged and plasma and tissue samples stored at −20°C prior to analysis. Plasma samples (10 μl) were precipitated with a mixture of 200 μl of acetonitrile and 20 μl of 35% perchloric acid containing 2 μg ml^{-1} of *p*-toluic acid as an internal standard. Samples were vortexed, centrifuged and supernatant assayed by HPLC. Tissue samples were minced with scissors and 100 mg added to 400 μl acetonitrile containing 2 μg ml^{-1} *p*-toluic acid with 20 μl 35% perchloric acid. Samples were vortexed, sonicated on ice for 30 s, centrifuged and the supernatant injected onto the HPLC.

Laser Doppler Measurements

Changes in cutaneous blood flow following application of the MeSA formulation to approximately 16 cm^2 area of forearm skin were monitored in a human volunteer. Skin surface probes (SP100) were attached to double-sided sticky discs over the application site and a dual probe Oxford Array Comprehensive Microvascular Assessment Unit (Oxford Optronix, Oxford, UK) used to continuously monitor blood flow at the treated and a control site on the same forearm.

HPLC 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. The methodology details are described fully in previous publications (11).

In Vitro Data Analysis

Flux (μg cm^{-2} h^{-1}) and permeability coefficient (cm h^{-1}) of MeSA and salicylate were calculated from regression lines fitted steady-state portion of cumulative concentration in the receptor chamber versus time plots for in vitro full-thickness skin diffusion studies. Where flux equalled the slope of the line/application area (cm^2), and permeability coefficient as flux/applied concentration. Lag times (T_{lag}) were approximated by solving this linear equation for y = 0.

In Vivo Data Analysis

The salicylate concentration in tissues below the topical application site were assumed to be a sum of directly penetrating salicylate and that from a redistribution of salicylate in the blood (9). It was further assumed that the salicylate concentration due to systemic redistribution is defined by salicylate concentrations in contralateral tissues (9). Hence, the salicylate concentrations due to direct penetration into tissues below the treated site are defined by the difference in the observed concentration and those in contralateral tissues. In allowing for differences in formulation bioavailability, the ratio of salicylate concentrations in any given tissue for different formulations should be identical, under conditions of identical blood flow, and equal to the ratio of the cumulative amounts delivered across the epidermis. Deviations in tissue ratios were therefore attributed to formulation blood flow effects. Areas under the tissue and plasma concentration-time curves from time of application to last sample collected were estimated by the model independent parabolas-through-the-origin (PTTO) method (12). The amount of unhydrolysed MeSA penetrating to each tissue, $F_{methylsalicylate}$ following application of the MeSA formulation was calculated from the AUC of MeSA divided by the AUC of total salicylates in each tissue. The ratio of the average total amount of salicylate (determined from the AUC for each tissue) in treated (Tr) and contralateral (Contr) tissues was calculated to estimate the bioavailability of the 2 formulations to compare to that seen in the in vitro studies.

RESULTS

In Vitro Studies

Table I shows the absorption parameters of MeSA and TSA applied to isolated rat skin. Approximately 25% of the

Table I. In Vitro Absorption and Skin Deposition of Methylsalicylate and Salicylate from Commercial Formulations Observed Using Full-Thickness Rat Skin

Formulation	20% methylsalicylate			10% triethanolamine salicylate
	Methylsalicylate	Salicylate	Total[a]	Salicylate
Flux ($\mu g\ cm^{-2}\ h^{-1}$)	47.5 ± 7.8	13.5 ± 2.3	56.6 ± 9.4	39.9 ± 11.8
Permeability coefficient($\times 10^{-3}$ cm h^{-1})	—	—	0.283 ± 0.05	0.798 ± 0.24
Estimated lag time (h)	3.9 ± 1.2	2.9 ± 1.1	—	3.2 ± 1.0
Amount absorbed in 24 h ($\mu g\ cm^{-2}$)	503.4 ± 88.1	372.9 ± 68.3	829.88 ± 148.3	1029.4 ± 170.3
Amount remaining in tissue ($\mu g\ g^{-1}$)	1699.1 ± 261.7	290.2 ± 35.7	1833.0 ± 273.3	653.5 ± 100.4

Note: Mean ± SEM, n = 3.
[a] Methylsalicylate + salicylate in salicylate equivalents.

MeSA absorbed through the skin was hydrolysed to salicylate. The steady-state flux of active ingredient (MeSA + salicylate) through the skin from the MeSA formulation (18% salicylate equivalent) appeared to be only 1.4-fold that from the TSA formulation (approx. 5% salicylate equivalent). After correcting for differences in applied concentration the resultant permeability coefficient of TSA was 3-fold higher than that of MeSA. At 24 h the total amount of salicylate penetrated through the skin was within 20% for the two formulations (Table I), reflecting the slower equivalent release through the dermal side must be occurring in the case of the MeSA formulation. The total amount of salicylate remaining in the skin at the end of the study was 2.8-fold higher following application of the MeSA formulation compared to the TSA formulation (Table I).

In Vivo Studies

Treated Tissue Sites

Figure 2 shows that substantially higher tissue levels of salicylate were found following in vivo application of the MeSA compared to the TSA formulation. The differences in tissue levels between the formulations, although expected to be higher for MeSA in the dermis because of higher retention seen in the in vitro studies, were much higher than could be explained by the 1.4-fold faster penetration seen in vitro. In addition to the increased tissue levels, an order of magnitude difference in the plasma total salicylate concentration-time profiles was also observed (Fig. 3). Levels of unhydrolysed MeSA in the plasma were low at only 2–3 $\mu g\ ml^{-1}$ throughout the study period. The concentration of unhydrolysed MeSA in tissues below the treated site is shown in Fig. 4A, where highest concentrations were observed in dermal and subcutaneous sites in the first hour of application.

Contralateral Tissues

In the contralateral tissues, as expected, salicylate concentrations following TSA application paralleled those in the plasma reaching a plateau around 4 h. In contrast, at 0.5 to 1 h after application of the MeSA formulation, there was a significant increase in concentration of total salicylate in the contralateral dermal tissue corresponding to 4–5 times above the circulating systemic plasma levels. At 2 h the dermal levels fell below the observed plasma salicylate concentration. The presence of unhydrolysed MeSA in contralateral tissues was

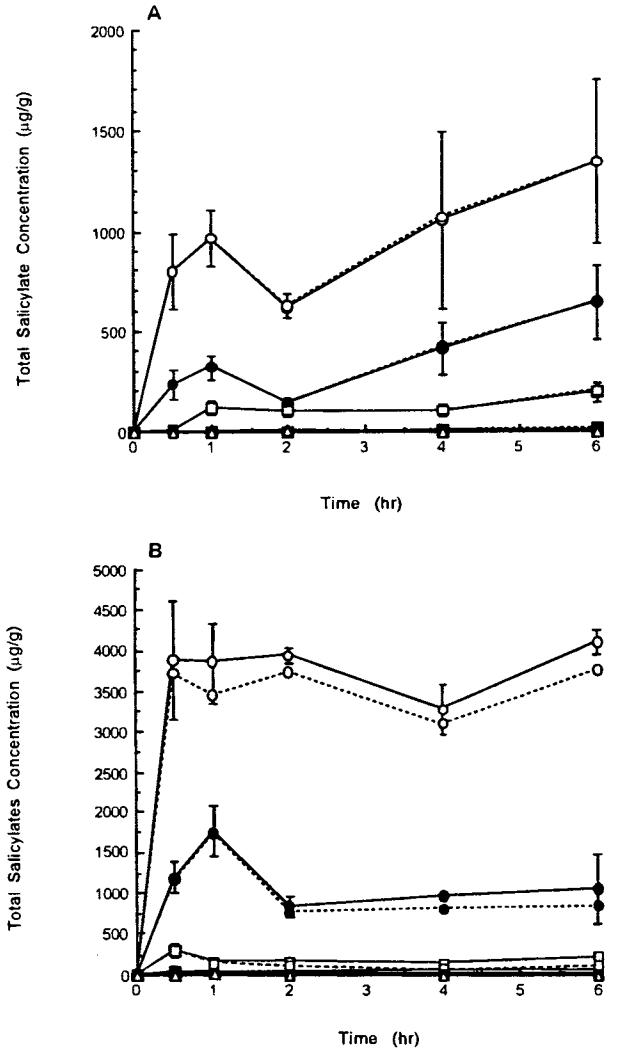

Fig. 2. Concentration-time profiles of total salicylate levels in rat tissues (dermis (○), subcutaneous (●), superficial muscle (□), deep muscle (■) and fat (△)) beneath treated sites following the application of (A) 10% triethanolamine salicylate and (B) 20% methylsalicylate formulations. Solid lines represent total salicylate levels observed at the treated site and dashed lines represent observed levels—levels observed in corresponding contralateral sites. Mean ± SEM, n = 3.

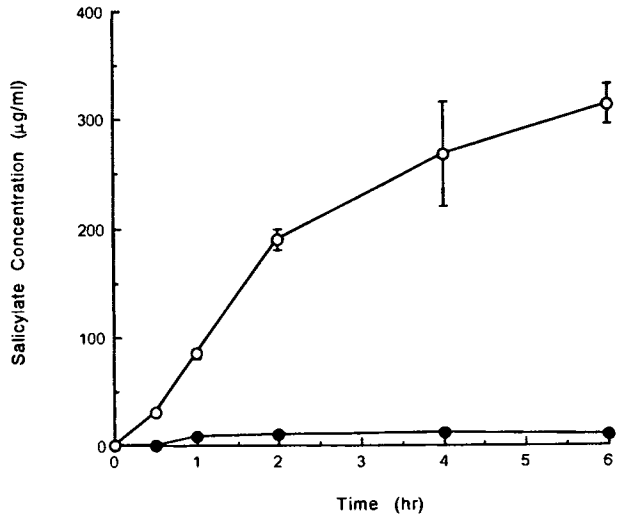

Fig. 3. Comparison of plasma concentration-time profiles in rats following application of 10% triethanolamine salicylate (●) or 20% methylsalicylate (○) formulation to a 9.6 cm^2 area of abdominal skin. Mean ± SEM, n = 3.

only observed at the 0.5 h time point (Fig. 4B). Table II also shows that the total fraction of MeSA observed in the tissues as a proportion of total salicylate varied from 0 to 0.26.

Laser Doppler

Consistent with the laser doppler probe being used in this work being calibrated for human use, we were unable to detect any significant blood flow response in our rat studies. The increases in cutaneous blood flow induced by MeSA in human topical application are shown in Fig. 5. This increase occurred within the first 0.5 h, coinciding with the rapid increases that were observed in the in-vivo rat tissue and plasma salicylate concentrations.

Table II also shows the ratios of the AUCs of the concentration-time profiles in each of the tissues for total salicylate from the topically applied MeSA and TSA formulations. The ratios average 3.86 and 26.44 for treated and contralateral tissue sites respectively, with plasma ratios similar to contralateral tissues at 23.95. Higher salicylate concentration ratios were seen in the deeper tissues below the site of application, whereas in the contralateral tissues the highest ratio was seen in the more superficial dermal regions. The ratio of tissue concentrations achieved by 'direct' penetration (treated-contralateral equivalent) averaged 2.75, slightly below the expected ratio of active ingredient in the two formulations, 3.78. It is apparent that large differences (normalised for the difference in concentration of active ingredient in the two formulations) exist between the formulations, with the greatest differences seen in favour of the MeSA formulation in plasma, subcutaneous tissue and deep muscle during the early phases of absorption (Fig. 2).

DISCUSSION

The present study has shown that different processes appear to dominate in the tissue distribution of topically applied salicylate following application in either its methyl ester or triethanolamine salt form. The stronger MeSA formulation was

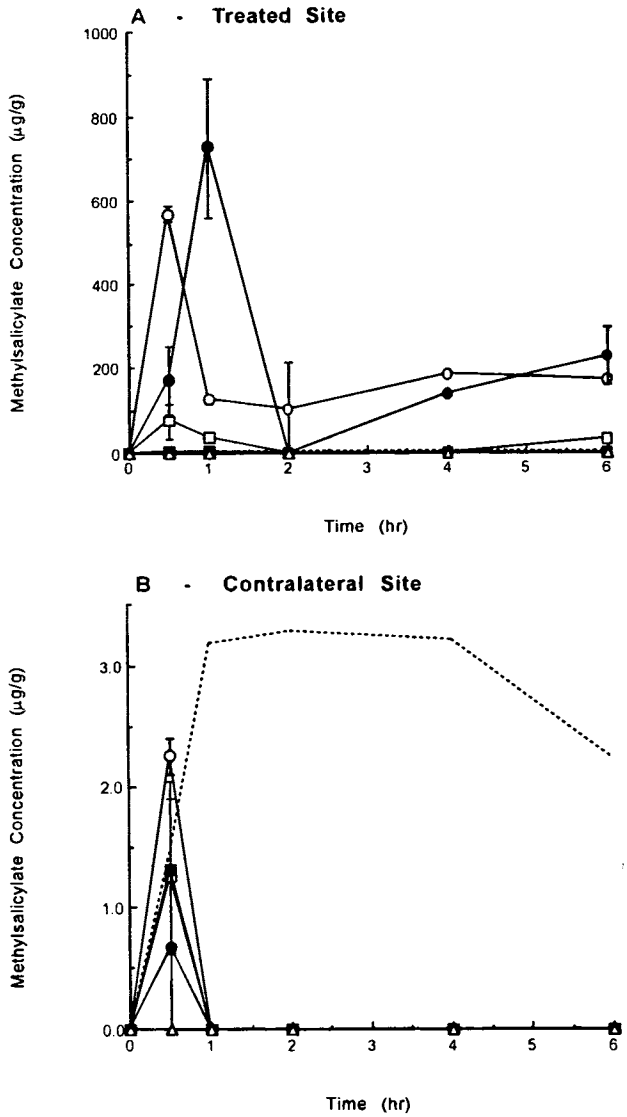

Fig. 4. Concentration of unhydrolysed methylsalicylate present in tissues below (A) the treated site and (B) in contralateral untreated tissues following application of the 20% methylsalicylate formulation (dermis (○), subcutaneous (●), superficial muscle (□), deep muscle (■), fat (△) with the dashed line representing plasma concentrations), n = 3.

more efficacious in achieving higher in-vivo levels of drug in underlying tissues compared to the TSA formulation, even above the ratio allowing for the difference in total salicylate concentrations in the two formulations, particularly in deeper tissues. Direct drug penetration into underlying tissues was seen with both salicylate formulations, consistent with the direct tissue penetration of topical TSA previously reported in a pig model where tissue concentrations were around 13 times that seen in plasma and 49 times contralateral tissue concentrations (8). In the present study, tissue: plasma ratios peaked for both formulations at the 0.5 h time point and were substantially larger for the TSA formulation, reflecting the much lower plasma concentrations observed with this formulation. It should be born in mind, however, that actual tissue:local plasma concentration ratios may be significantly lower than those observed using systemic plasma levels, due to the effects of dilution in the whole circulating blood volume.

Table II. Analysis of Relative Amounts of Methylsalicylate and Total Salicylate Present in Tissues Following Application of Two Salicylate Formulations

Tissue	$F_{methylsalicylate}$[a]	In vivo ratio[b]		
		Treated	Contralateral	Direct[c]
Dermis	0.05	3.97	45.80	3.74
Subcutaneous	0.20	2.93	15.83	2.68
Superficial muscle	0.26	1.50	23.24	0.97
Deep muscle	0.06	8.46	20.9	2.02
Fat	0.00	10.59	—	4.32
Mean	0.10	3.86	26.44	2.75
SEM	0.04	1.73	6.64	0.60
Plasma	0.01		23.95	

[a] AUC methylsalicylate/(AUC methylsalicylate + AUC salicylate).
[b] AUC total salicylate after methylsalicylate/AUC salicylate after triethanolamine salicylate.
[c] Direct = penetration into tissue-equivalent penetration into contralateral tissue. Data represents the ratio of average AUC calculations.

The tissue salicylate concentration-time profiles observed in-vivo did not appear to correlate with the suggestions of the in-vitro findings, with substantially higher concentrations of salicylate seen following application of the MeSA formulation than were predicted, particularly with levels over 20-fold that seen with TSA in the muscle areas during the first hour. Consistent with this observation, in a recent study using cutaneous microdialysis we demonstrated significant dermal and subcutaneous tissue concentrations of salicylate following application of an identical MeSA formulation compared to negligible concentrations detected following application of TSA in human volunteers (5). Within 0.5–1 h of applying both test formulations in vivo, substantial amounts of salicylate were observed in tissues underlying the application site, with plasma concentrations rising rapidly during this time. This data suggests that the biological processes lacking in the in vitro study design are dominating the absorption process in vivo, the most significant being the involvement of the local blood supply.

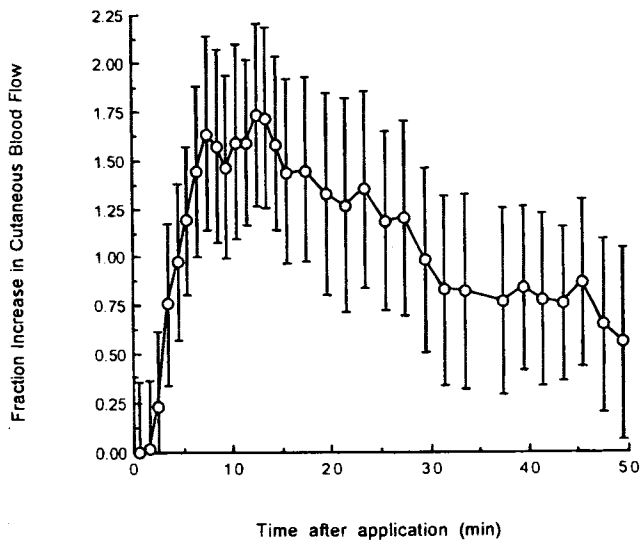

Fig. 5. Fraction increase in human cutaneous blood flow assessed using laser doppler flowmetry following the application of the identical commercial MeSA formulation used in the present study. Mean ± SD 30 s–1 min reading.

In the present study we can see from the difference in plasma concentrations and the treated tissue-contralateral tissue concentrations presented in Fig. 2 and Table II, that a greater component of drug penetrating from the MeSA formulation is carried and distributed in the systemic circulation compared to the TSA formulation. Despite this clearance, underlying tissue concentrations ($\mu g\ g^{-1}$) of salicylate from the ester formulation were consistently higher than contralateral sites, more substantially in the first hour following application, at depths to which diffusion alone could not have accounted for the concentrations observed. This suggests that there could be a local enhancement of drug distribution into deeper tissues by the local dermal blood supply. The importance of the systemic circulation in the distribution of topically applied drugs to tissues beyond the application site is clearly shown in this study and has been previously recognised in man (6,7). The rapid clearance of topically applied drugs into the dermal circulation is usually considered undesirable when targeting tissues below the application site. However, the orientation of local blood vessels could also help in the rapid distribution of drugs picked up from the epidermal dermal junction and carried down into deeper tissue sites. McNeill *et al.* (13) first suggested that the orientation of local blood vessels from the surface down into muscular sites beneath the site of application of topical drugs enhanced their deep tissue penetration compared to application over areas which did not contain such complicated vascular networks. In addition, in examining the distribution of dermally applied solutes in a perfused rat hindlimb model, we have found tissue distribution patterns which could not have been due to diffusion alone and further suggested that orientation of local vascular networks may contribute to the tissue distribution of topically applied drugs (14).

The enhanced distribution of unhydrolysed MeSA seen in the first 0.5–1 h following its application is consistent with its known ability to increase local blood flow, and thus its own clearance into the plasma. Though we have no direct measurement of blood flow changes in rats, our indirect evidence from humans together with literature data on the effects of MeSA on cutaneous blood flow in humans and mice, supports an increase in blood flow following MeSA application. In humans feelings of local "warming" or "burning" are the dominant sensations reported following the application of MeSA in concentrations above 3% (15). This study has shown that maximal changes in human cutaneous blood flow, with the same MeSA formulation used in the present study, occur in the first 0.5 h following application (Fig. 5). This finding is also consistent with the rapid effect of MeSA suggested in our previous studies in which dermal and subcutaneous levels of salicylate, estimated in humans using cutaneous microdialysis, rose more rapidly than could be accounted for by diffusion alone following application of the MeSA formulation (5,16). In addition, Patrick *et al.* (17,18) demonstrated that the cutaneous response to topically applied MeSA was primarily vascular, with both vascular permeability and blood flow significantly increasing within minutes of application and maintained for 20 min compared to vehicle treated ears in mice.

In contralateral tissues, unhydrolysed MeSA was only detected in the first 0.5 h, which coincides with the maximum increases in cutaneous blood flow and represents the period

over which unhydrolysed ester would be capable of being rapidly cleared into the circulation with shorter exposure time to skin esterases. The hydrolysis of MeSA in the blood is reportedly around 25-fold lower than in the skin (19), giving it a much longer half life and the ability to distribute in detectable concentrations into contralateral tissues when plasma concentrations are sufficiently high. The higher relative fraction of MeSA in subcutaneous tissue and superficial muscle beneath the application site at this time point (Fig. 4A) could reflect a combination of lower enzymatic activity in these areas and a more favourable partitioning of MeSA into these tissues over the more water soluble hydrolysed salicylate.

Consistent with the present study, high circulating plasma levels of salicylate have been reported in man following topical application of MeSA compared to other salicylate salts. Morra *et al.* (4), in their comparison of salicylate and metabolite plasma concentration-time profiles and urinary excretion data following application of TSA or MeSA to the thighs of human volunteers, found that plasma levels of salicylate from the triethanolamine salt formulation were below their assay detection limit and urinary excretion within the first 24 h over 25-fold lower compared with the MeSA formulation. Additionally, Danon *et al.* (20) showed that increases in skin blood flow and temperature associated with heat and exercise contributed towards a 3-fold enhancement in topical absorption of MeSA reflected by plasma concentration-time profiles and urinary excretion data. Heng (21) also reported that local tissue toxicity of MeSA and menthol, resulting in local skin and muscle necrosis and persistent interstitial nephritis, was observed in a patient applying the topical formulation in combination with use of a heating pad. The increased blood flow and possible solute diffusion kinetics as a result of the application of the heating pad causing significantly raised skin and underlying deeper muscle salicylate concentrations in this patient. Raised plasma salicylate following application of topical MeSA has also been highlighted as a potential danger in patients receiving anticoagulant therapy with warfarin, with several cases of potentiation of warfarin activity reported normally only expected in conjugation with systemic nonsteroidal antiinflammatory drug treatment (22–24).

One of the aims of determining the kinetics of absorption of these topical salicylate formulations was to determine whether pharmacologically active concentrations of salicylate are achieved in muscle layers or other deep tissue sites targeted below the site of application. The actual tissue concentrations of salicylate needed to effectively inhibit cyclooxygenase activity are difficult to ascertain from the literature. In a study by Higgs *et al.* (25), examining plasma and subcutaneous carrageen-containing sponge inflammatory exudates in rats dosed orally with aspirin, reported that inflammatory exudate salicylate levels of around 130–150 $\mu g\ ml^{-1}$ were required to reduce the production of prostaglandin E, one of the primary inflammatory mediators, by 50%. These exudate levels corresponded to plasma salicylate concentrations of around 160 $\mu g\ ml^{-1}$ (25). In the present study, plasma salicylate levels following application of the MeSA formulation reached over 300 $\mu g\ ml^{-1}$ whilst those following TSA application only around 10 $\mu g\ ml^{-1}$. Tissue concentrations below the application site were well within the predicted therapeutic range in dermis and subcutaneous tissue for both formulations throughout the 6 h time series studied. In deeper tissues, the ester formulation produced anti-inflammatory concentrations in superficial muscle over the 6 h with the triethanolamine formulation only achieving concentrations greater than 130 $\mu g\ g^{-1}$ at 6 h. In deeper tissues, concentrations of salicylate ranged between 40–69 $\mu g\ g^{-1}$ and 1–13 $\mu g\ g^{-1}$ in deep muscle and 6–25 $\mu g\ g^{-1}$ and 0–5 $\mu g\ g^{-1}$ in fat for the MeSA and TSA formulations respectively. This data suggests that neither formulation may be capable of providing sufficient anti-inflammatory activity in the deeper tissue compartments, but that the ester formulation is capable of achieving rapid and prolonged therapeutic effect in superficial muscle tissues.

In conclusion, the present study has shown that tissue and plasma concentrations of salicylate following application of MeSA rise rapidly within the first hour of application and that this effect is mirrored by indirect evidence of its effects on blood flow that we observed in humans and that reported by other groups in humans and mice (15,17,18). We have demonstrated that direct tissue penetration of salicylate does occur below topical application sites from both ester and salt formulations but that tissue concentrations achieved are higher than predicted following MeSA application due to its suggested rapid distribution to these sites in the blood. We have suggested that, based on predictions of minimum therapeutic concentrations, the actual therapeutic benefit of the salicylate levels determined in underlying tissues may not be sufficient at depths below the superficial muscle in the animal model used, though this remains to be further elucidated in humans. We also noted that in-vitro isolated skin penetration rates do not necessarily reflect in-vivo drug absorption when processes such as changes in perfusion and clearance by the local blood supply play an integral part in the distribution of solutes.

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