Radial Spread of Sodium Lauryl Sulfate After Topical Application

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Purpose. Since topical application of sodium lauryl sulfate (SLS) has been reported to elevate transepidermal water loss and decrease skin capacitance in areas immediately adjacent to the applied site, studies were carried out to quantify the extent of radial spread of SLS below a topically exposed site in a hairless rat model.

Methods. Fixed sites were demarcated and the levels of SLS measured around the applied site in the epidermis, dermis and the subcutaneous tissues. Underlying deep tissue penetration and radial spread of SLS in the presence and absence of a vasoconstrictor, phenylephrine, was also quantified.

Results. In a typical 24 hour study, the radial spread of SLS was observed to a distance of ~ 0.75 cm from the applied site. The use of phenylephrine (1:20000), did not significantly enhance either the local underlying tissue (apart from underlying epidermis) concentration or radial spread of SLS relative to no vasoconstrictor treatment.

Conclusions. Given that SLS impairs barrier function of the skin, its radial spread could be explained by a passive diffusion process. Vasoconstrictor did not remarkably alter SLS penetration and radial spread possibly due to the competing effects of vasodilation (caused by SLS) and vasoconstriction (caused by phenylephrine).

KEY WORDS: topical SLS application; radial tissue distribution; passive diffusion; cutaneous clearance.

INTRODUCTION

SLS is a surface active agent with skin penetration enhancer properties[1,2]. At higher concentrations SLS affects both keratin proteins and lipids in the keratinocytes causing prolonged damage to the skin barrier function[3,4]. Standard patch application of SLS under occlusion also increases the local blood flow, decreases skin surface hydration, and elevates transepidermal water loss[5-9]. In a recent report we observed that topical application of SLS increased transepidermal water loss and skin dehydration at the adjacent, unexposed sites[6]. This phenomenon was attributed to the radial spread of SLS below the applied site[6]. Radial transport of topically applied methyl nicotinate has also been demonstrated[10,11]. Underlying deep tissue penetration of SLS below a topically applied site[20] has also been quantified.

During the current investigation, radial spread of SLS in underlying epidermis, dermis and subcutaneous tissues was quantified in vivo in a hairless rat model after 6h, 24h and cumulative (two applications of SLS solution for 24 h each, with an interval of one week between the applications) topical applications. In vitro experiments were also conducted with excised rat skin to gain an insight into the mechanism of radial spread of topically applied SLS.

Local vasoconstrictors such as epinephrine and phenylephrine (PE) are often administered locally to decrease systemic absorption of local anesthetics[12,13,14]. Phenylephrine is a synthetic vasoconstrictive agent and may be preferred over epinephrine because it has no direct cardiac effects[12]. The use of PE has been shown to elevate local tissue concentrations of a number of compounds with decreased systemic uptake[14]. The effects of PE on underlying and radial tissue distribution of SLS were also a subject of the present study.

MATERIALS AND METHODS

Chemicals and Instrument. [^{35} S] Sodium lauryl sulfate (> 95% purity) was purchased from Amersham International, Illinois, USA; tissue solubilizer-Soluene-350 and Insta-flour for organic samples were obtained from Packard Instrument Co., Meridan- Connecticut, USA and the cocktail Universol for inorganic samples from ICN-CA, USA. Sodium lauryl sulfate and phenylephrine hydrochloride were obtained from Sigma Chemical Co., St. Louis, Missouri USA. Other reagents used were of analytical grade. A liquid scintillation counter (Packard Instrument Co., Model Tricarb-1500) was used to detect the radioactivity in the samples.

Animals. Hairless rats (350-400 g) were obtained from the Charles River Laboratory. The animals were housed under standard laboratory conditions 25.0 ± 0.5 ° C, relative humidity 55-75% and supplied with normal pellet diet and water ad lib. All the experiments were approved by the Committee for Animal Research, University of California- San Francisco.

Patch Test Treatment. Standard patches (Large Hilltop chambers- Cincinnati, OH; inner diameter 1.5 cm) were applied on the dorsum of the anesthetized animals. The patches were secured in place with the help of an adhesive tape (3M Transpore Surgical tape, Minnesota, USA) and an elastic bandage. The animals were divided into 4 study groups with each group receiving one of the following treatments:

a. single application of 1% aqueous SLS solution for 6h (300μl).

b. single application of 1% aqueous SLS solution for 24h (300μl).

c. Two applications of 1% aqueous SLS solution for 24 h each. The time interval between the two applications was one week (300μl).

d. single application of 1% aqueous SLS (300μl) + 0.0002% phenylephrine for 24h.

Each topically applied patch approximately contained 2μCi of radiolabelled SLS.

In Vivo Dermal Penetration and Local Tissue Uptake Studies. These studies were only conducted for treatments b and d as stated above. The data for underlying deep tissue penetration for other treatments viz. 6h and cumulative treatments has been communicated elsewhere[20]. After dosing under anesthesia, the animals were allowed to recover and then placed in separate cages. At predetermined time (24h post dosing, in the presence or absence of PE) the

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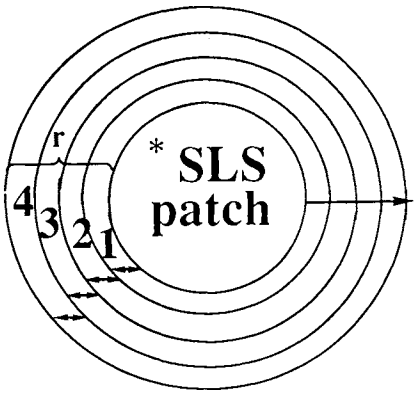

Fig. 1. Schematic diagram illustrating demarcation of the radial tissues around the site of SLS patch application.

animals were lightly anesthetized using anesthetic ether and the blood withdrawn from the tail vein in heparinized tubes. The blood samples were centrifuged immediately at 2000 rpm to separate the plasma. A known amount of plasma was taken in the scintillation vials and the scintillant added to assess the SLS activity in the plasma.

The animals were then sacrificed by an overdose of anesthetic ether, the patches removed carefully and the test area swabbed dry with tissue paper. A portion of the test area was tape stripped 15 times to remove the stratum corneum. The scintillant was added to the tape stripped samples and counted for radioactivity. The skin was excised and epidermis and dermis separated by heat splitting at 60° C in a water bath. The underlying subcutaneous tissue, fat, muscle and deep muscle layers were dissected out surgically. Similar procedure was adopted to dissect the corresponding tissues from the untreated contralateral side. To avoid radioactivity contamination, surgical instruments were thoroughly cleaned and wipes taken before each tissue excision. The tissues were then weighed, put in the vials containing tissue solubilizer and kept in an incubator at 45° C overnight to dissolve the tissue. Scintillant cocktail was added into these vials and the radioactivity counted in the scintillation counter. Background radioactivity was determined for plasma and various tissues in one control animal.

Radial Tissue Excision. Four different regions (r-1, r-2, r-3 and r-4), each being approximately 2.0 - 2.5 mm in diameter were demarcated around the test site and dissected out in concentric circular pieces using a surgical blade (Fig. 1). Tissues were dissected in the order starting from the periphery and moving towards the patch site (from r-4 to r-1) to avoid contamination from one radial site to another. The tissues were treated similarly as described above to detect the radioactivity in each tissue sample. The radial spread studies were carried out for all treatments (6h, 24h, cumulative and co-application of a vasoconstrictor).

In Vitro Radial Spread Studies. Skin from euthanized rat was excised and placed in a petri dish containing tissue paper swabbed in MEM Eagle with Earle's BSS culture medium (obtained from UCSF Cell Culture Facility). A single patch of 1% SLS was applied in the middle of the excised skin and kept in the water bath at 37° C for 24h. At 24h post application, the patch was removed and the dissection and estimation of SLS in different skin layers and the radial sites was carried out as described above for the in vivo studies (Fig. 1).

Calculation Method. The dpm in the tissues (after correction for background counts) were converted to fraction of initial applied concentration and expressed as μmoles/g of the tissue. Statistical comparisons were made using paired or unpaired Student's t test where appropriate. The level of significance was taken as $p = 0.05$.

RESULTS AND DISCUSSION

Effects of PE on Deep Tissue Penetration of SLS. The distribution of SLS in the stratum corneum tape-strips was comparable ($p>0.05$) in the presence and absence of phenylephrine (Fig. 2).

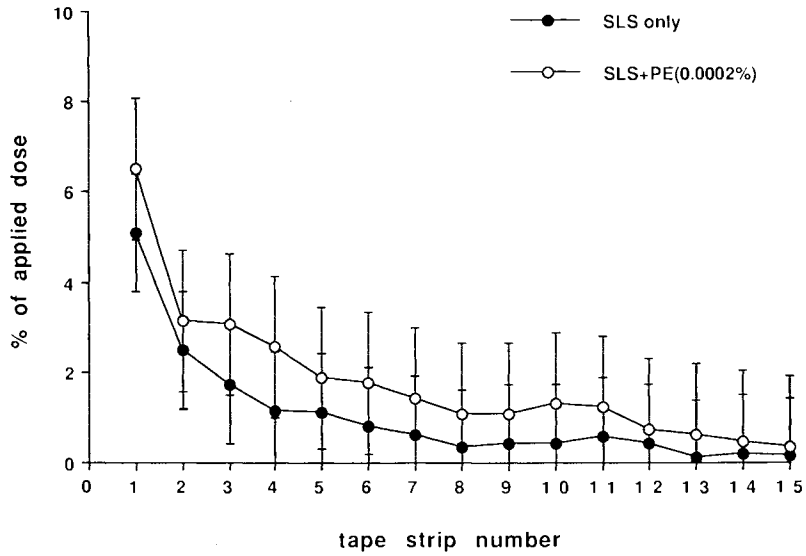

Fig. 2. SLS distribution in stratum corneum expressed as percent of applied dose. Values expressed as mean ± SD (n=4).

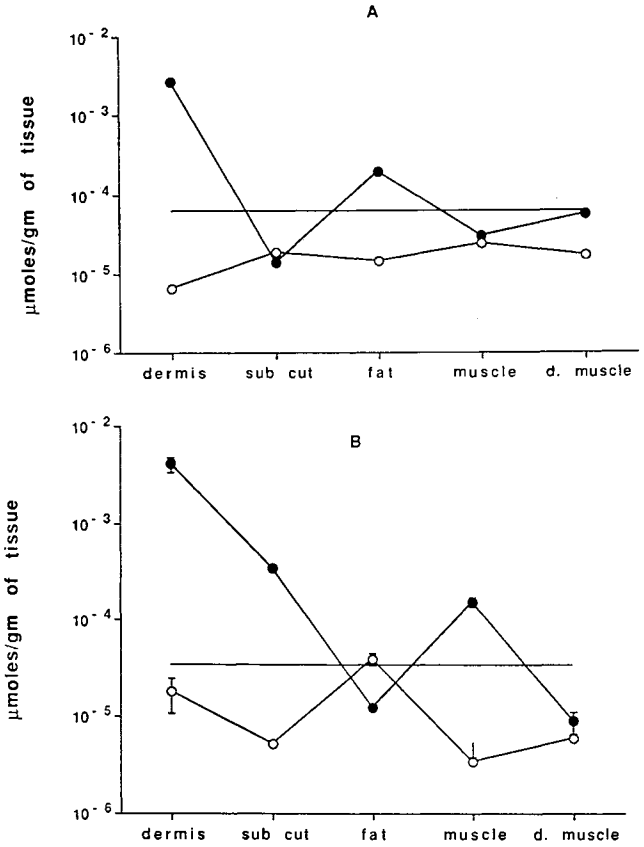

Fig. 3. Tissue distribution of SLS in the presence (A) and absence (B) of PE after 24h topical application. (●) underlying tissues; (○) contralateral tissues; (—) blood. Values expressed as mean ± SD (n=4). sub cut= subcutaneous tissue, d.muscle=deep muscle.

Figs. 3A and 3B show the underlying and contralateral tissue concentration-depth profiles of SLS in the presence and absence of PE. SLS concentration in the underlying epidermis in the presence and absence of PE treatments was 20.93 ± 2.8 and 6.33 ± 1.80.µmoles/gm of tissue respectively. Underlying epidermis and dermis (and subcutaneous tissue in the absence of vasoconstrictor) had higher concentrations as compared to blood and corresponding contralateral tissues indicating direct penetration of SLS to the depth of ~ 5-6 mm below the applied site. The concentrations of SLS in tissues deeper than subcutaneous tissue (except for muscle in the absence of vasoconstrictor) were comparable to plasma and contralateral tissue concentrations ($P>0.05$) suggesting systemic redistribution of SLS.

The plasma levels in the presence (0.004±0.002 µmoles/gm plasma) and absence of PE (0.006±0.003 µmoles/gm plasma) were comparable ($p>0.05$). Co-administration of PE significantly enhanced SLS concentration in underlying epidermis relative to no vasoconstrictor treatment ($p<0.002$). Underlying dermis showed comparable concentrations in the presence and absence of PE. The observed levels of SLS in underlying subcutaneous tissue ($p<0.0001$) and muscle ($p<0.0001$) were significantly higher (while deep muscle ($p<0.0001$) and fat ($p<0.001$) observed lower values) in the absence of PE as compared to the concentrations in the presence of PE. The application of local vasoconstrictors has earlier been shown to elevate the underlying deep tissue penetration of a number of solutes[14] and also prolong the duration of local response[12]. PE, being a local vasoconstrictor, reduces local dermal blood flow[14], thereby allowing solutes to diffuse into underlying tissues. Consistently, higher levels of SLS after PE treatment in all the underlying tissues were not observed in this study. Topical application of SLS has local vasodilatory effects[2]. In this case where SLS and PE were coapplied the local vasoconstricting effects of phenylephrine in the deeper layers may be offset due to the competing mechanisms of vasoconstriction and vasodilation occurring almost simultaneously and possibly mediated through the same receptors. The extent of this competing mechanism in dermis and other tissues cannot be estimated from the present study. The levels of PE in dermis and underlying tissues were not quantified in the present work.

Radial Uptake of SLS. The radial spread of topically applied SLS in underlying tissues was evaluated by comparing the radial tissue concentrations of SLS with concentrations in corresponding tissues on an untreated contralateral site. For all treatments r-4 SLS concentrations in epidermis, dermis and subcutaneous (except for 24h, $p=0.009$) tissues were comparable ($p>0.05$) to contralateral tissue concentrations suggesting systemic redistribution (Fig. 4A-D).

SLS concentrations in r-3 epidermis, dermis and subcutaneous tissues were comparable ($p>0.05$) to contralateral sites for all treatments except for 24h treatment where r-3 epidermis ($p=0.03$) and r-3 subcutaneous tissue ($p=0.001$) showed significant elevated SLS levels (Refer fig.4A-D). r-3 epidermal levels of SLS after PE treatment were also higher than the contralateral site ($p=0.01$). r-2 SLS concentrations were significantly higher than the contralateral tissue concentrations for epidermis (24h-$p=0.02$; 6h, $p=0.04$; PE treatment, $p=0.01$); dermis (24h, $p=0.03$; 6h, $p=0.006$; cumulative, $p=0.05$) and subcutaneous tissue (24h, $p=0.01$; 6h, $p=0.001$). r-1 SLS concentrations were again significantly higher than the corresponding contralateral concentrations for epidermis (24h, $p=0.004$; 6h, $p=0.03$; cumulative, $p=0.05$; PE treatment, $p=0.003$); dermis (24h, $p=0.01$; 6h, $p=0.02$; cumulative, $p=0.03$ and PE treatment, $p=0.03$) and subcutaneous tissue (24h, $p=0.02$; PE treatment, $p=0.02$). These results suggests that radial outspread of SLS from below the applied site is limited to a maximum distance of r-3 i.e. around 0.75 cm for a 24 hour study. Previously, physical parameters such as transepidermal water loss and capacitance were observed to be higher than normal in the area adjacent to the site where SLS was applied. The present results quantitatively reinforce the phenomenon of radial spread of SLS proposed in that report[6].

In Vitro - in Vivo Comparison of Radial Spread (Same Conc. Duration and Area). Higher concentrations of SLS were observed in vitro in the underlying tissues (Fig.5) as well as in the radial dermal and subcutaneous tissues (r1-r4) as compared to the in vivo concentrations (Fig.6B-C). The lack of an adequate clearance mechanism due to the absence of viable dermal microcirculation in vitro is responsible for the observed high concentrations of SLS in the underlying and radial tissues. Given that epidermis is an avascular tissue, the SLS concentrations were more comparable (in contrast to dermis and subcutaneous tissue) in all the radial tissues (r1-r4) after in vitro and in vivo treatments (Fig. 6A).

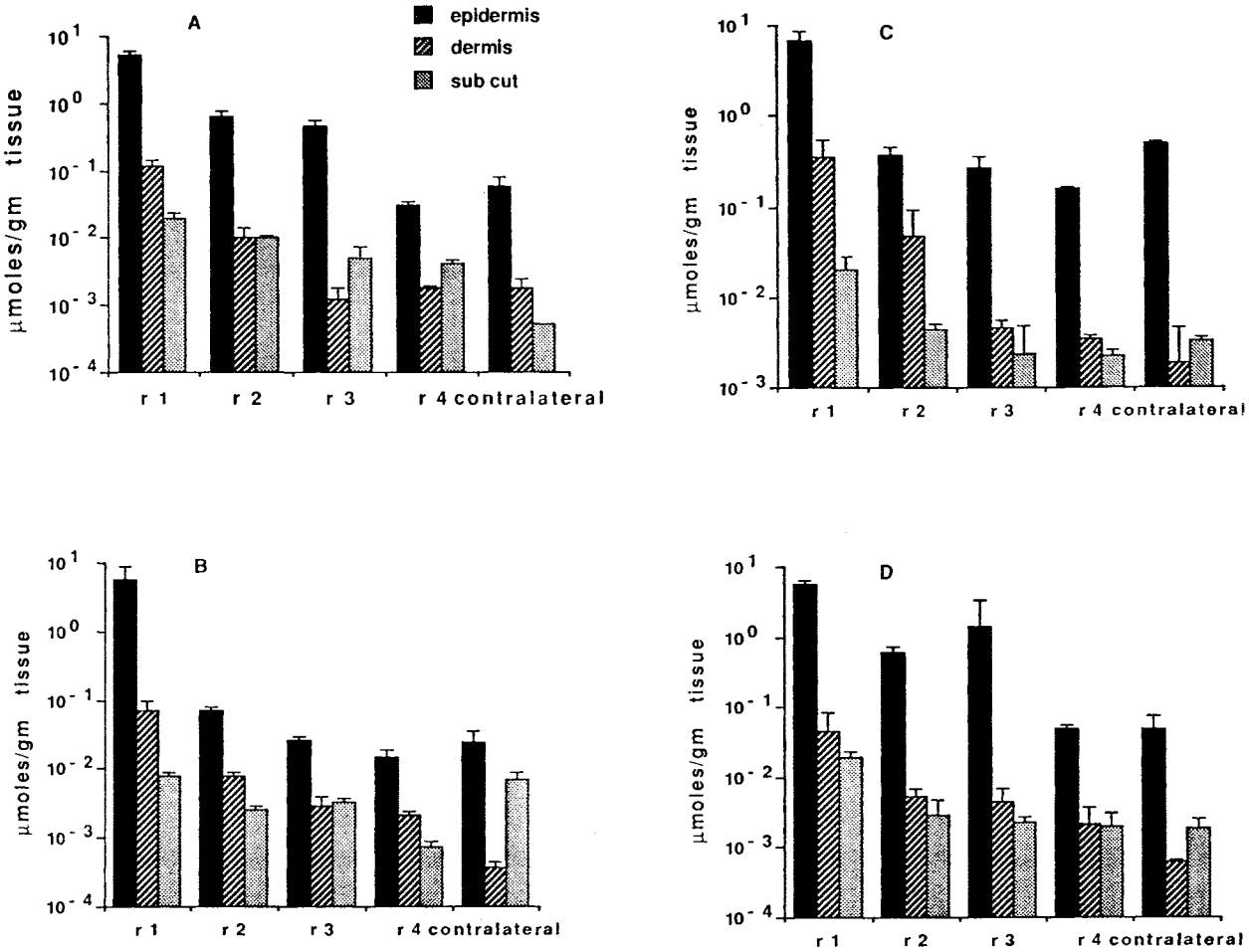

Fig. 4. Underlying radial tissue distribution of SLS after topical application. A = 24h, B = 6h, C = cumulative and D = phenylephrine treatment. Values expressed as mean ± SD (n = 4).

The absence of the viable capillary network in in vitro studies and the observed significant radial spread in vitro suggest that the radial spread of SLS occurs by a simple passive diffusion phenomenon.

The importance of dermal microcirculation in the radial transport of methyl nicotinate has earlier been suggested[10]. Radial spread of methyl nicotinate was observed to a distance of 1 cm after only 10 min. topical application in human volunteers. The observed radial spread of methyl nicotinate was interpreted in terms of the erythematous vasodilatory response below the applied site. A capillary/dermal tissue exchange of methyl nicotinate flowing away from the applied site was proposed to account for such a rapid radial spread of methyl nicotinate[10]. This mechanism was further fitted to a theoretical model by Albery et al[11].

In the present case, it can be seen that SLS is transported radially to a distance (h) of ~0.25 cm after a time (t) of 6h patch application. By applying Einstein-Smoluchowski equation[5,10], $t = h^2/2D$ (D is the diffusion coefficient in cm^2/sec), an apparent diffusion coefficient of the order of 10^{-6} cm^2 /sec can be estimated. This value is consistent with an average aqueous diffusion coefficient for comparable sized molecules[16] but much higher than the average diffusion coefficient of 10^{-9} cm^2/sec for human stratum corneum[17] (for

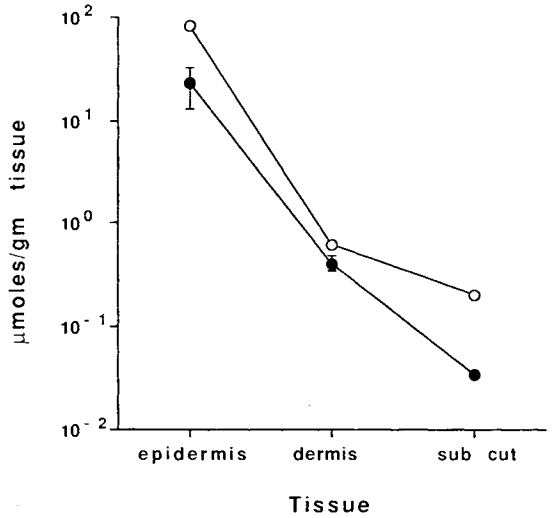

Fig. 5. Underlying tissue distribution of SLS after 24h topical application. (○) In vitro treatment (n = 1); (●) In vivo treatment (n = 4).

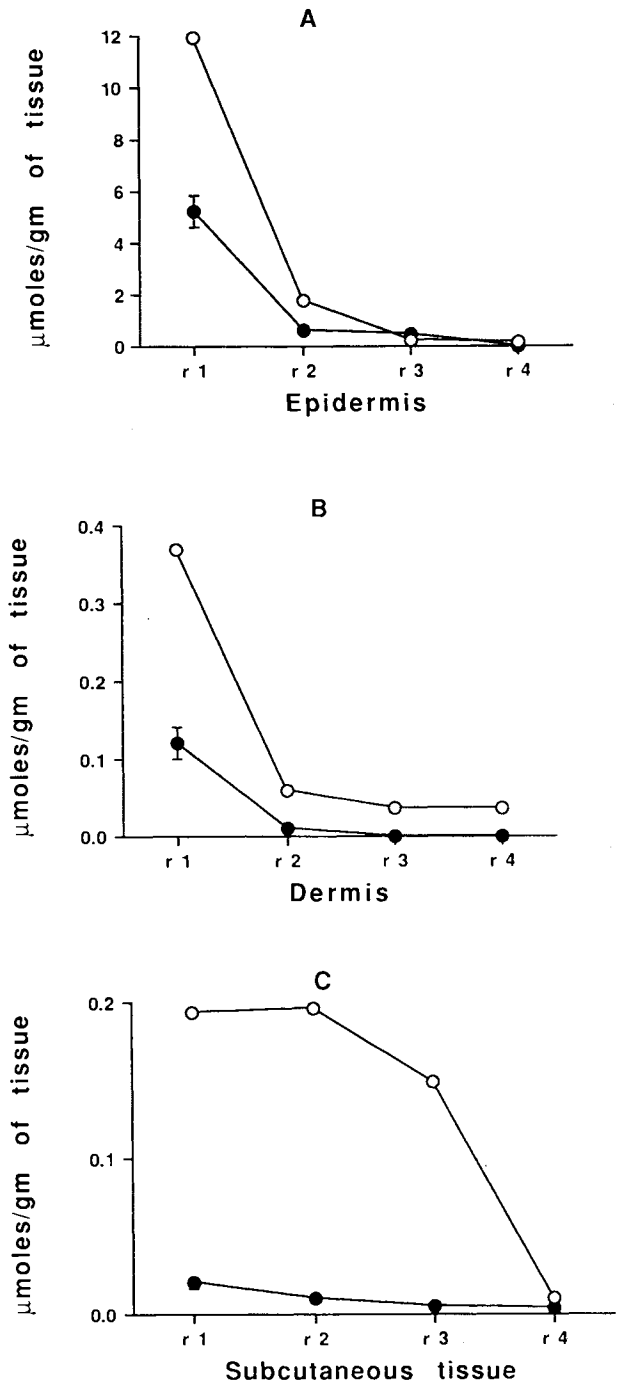

Fig. 6. Underlying radial tissue distribution of SLS after 24h topical application; (○) In vitro treatment (n=1); (●) In vivo treatment (n=4).

solutes which do not interfere with the skin barrier properties). SLS is known to act as a penetration enhancer by possible fluidization of epidermal lipids[3,8], the action depending on the applied concentration and duration of application[3-5,18]. High concentrations are even known to extract skin lipids[7-9]. The epidermal barrier disruption effects of SLS coupled with possible increase in lipid fluidity below the applied site may allow it to diffuse rapidly in all directions including the radial path. The apparent radial spread in dermis is understandable given the relatively aqueous composition of the tissue. Thus, on the evidence available, it is suggested that radial penetration of SLS can be accounted for by passive diffusion without needing to invoke other mechanisms. The possibility of subtle variations in the radial diffusion process for specific skin layers was not investigated in the present study.

Comparable levels of SLS were observed in r-1, r-2 and r-3 epidermis in the presence and absence of PE ($P>0.05$); the concentrations of SLS in radial dermal tissue were also comparable ($p>0.05$) in the presence and absence of PE for r-1 and r3 tissue (r-2 dermis ($p=0.02$) noted high levels of SLS in the absence of PE) (Fig.4A&D). r-2 and r-3 subcutaneous tissues also demonstrated elevated levels of SLS in the absence of PE (r-2, $p=0.01$; r-3, $p=0.03$). The use of vasoconstrictor did not yield the expected high levels of SLS in the radial tissues probably due to the opposite effects of the vasoconstrictor, PE, and the vasodilator, SLS, as discussed earlier. The insignificant effects of PE observed in this study should not be interpreted as to the lack of vasoconstricting properties. Co-penetration and the resultant vasoactive effects of SLS and PE on the skin and deeper tissues after topical application cannot be deciphered from the present results.

Taken together, these experiments for the first time quantitate the radial spread of SLS below a topically applied site. The observed sensitivity of the unexposed area adjacent to SLS application reported previously may thus be attributed to its radial spread. This radial spread of SLS may be explained by the simple diffusion process. The pharmacologic and toxicologic implications of this data are extensive. The above studies should be performed with other compounds being used or under investigation for transdermal and topical therapy as well as for penetration enhancers to evaluate the possibility of skin sensitization of unexposed areas adjacent to the exposed sites. The study may also have implications in the choice of skin site for patch or other topical product therapy particularly with repeated applications. Further parameters such as concentration, area of the patch and duration of exposure need to be studied to better understand the phenomenon of radial spread.

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