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Effect of Topical Laurocapram (Azone®) on the *In vitro* Percutaneous Permeation of Sodium Lauryl Sulfate Using Human Skin

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The probability of simultaneous cutaneous exposure to surfactants and penetration enhancers could occur frequently during routine skin treatment. This study ascertains whether pre-exposure of skin to laurocapram would affect the penetration of a model surfactant, sodium lauryl sulfate (SLS). In vitro experiments with human skin were performed to compare the penetration of SLS after pretreatment with (1) different concentrations of laurocapram, (2) after repeated SLS treatments, (3) untreated controls, and (4) water-control. Pre-exposure to laurocapram enhanced penetration of SLS compared to all other treatments ($p<0.05$). Since subsequent pre-exposure of skin to laurocapram increased SLS penetration, the chances of an elevated skin irritation reaction at the exposed site may therefore be possible. Pre-exposure of the skin with SLS did not increase the SLS flux values significantly, compared to the laurocapram pretreated skin. From these results it can be proposed that proper care and precautions may be necessary after exposure of skin to laurocapram and also to various other percutaneous enhancers. Further in vivo correlations are essential to define the clinical implications of this study, especially as related to irritant dermatitis. ***Key words: percutaneous penetration; skin irritancy; penetration enhancer; topical application.***

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Sodium lauryl sulfate (SLS) is an anionic, amphiphilic surfactant, extensively used as a biological tool in various consumer products and for industrial purposes. Due to its widespread topical use, the skin is often exposed at length to SLS. In 'every day' life the contact is usually cumulative and of short duration (hand, or dish washing), due to which irritation may occur in susceptible individuals.

Laurocapram, an N-alkylated cyclic amide, has proven to be an effective penetration enhancer for the percutaneous delivery of certain topically applied drugs and has been successfully formulated into gels, creams and lotions with both lipophilic and hydrophilic compounds. The penetration-enhancing abilities are dependent on (1) penetrant lipophilicity or hydrophilicity and (2) laurocapram concentration; concentrations in the range of 1% to 10% are most effective (1, 2).

Both SLS (3, 4) and laurocapram are known to alter the skin barrier function by their action on the epidermal lipids and keratin proteins (5–7). The effect of laurocapram on the stratum corneum lipid structure may be due to its partitioning into the liquid crystalline phase and forming reversed type phases, thus altering the barrier and increasing permeation of drugs (7–9). Since both substances are likely to be employed alternatively/simultaneously onto the skin, an accentuation of SLS-caused skin reactions by laurocapram would not be impossible. The present in vitro study was therefore conducted to investigate whether (1) pre-exposure of skin to laurocapram increased the permeability of SLS into the skin, (2) different concentrations of laurocapram had a differential effect on permeation of SLS, and (3) repeated exposure to SLS had a similar effect to that of laurocapram pretreatment on SLS permeation.

MATERIAL AND METHODS

Materials

[^{35}S]-SLS was purchased from Amersham Company, IL, USA, laurocapram (Azone®) from Discovery Therapeutics, Inc. (purity, 99.3%), Richmond, VA, USA, and the scintillation cocktail (Universol) from ICN- CA, USA. Other chemicals and solvents were of reagent grade and obtained commercially. A liquid scintillation counter (Packard Instrument Co., Model Tricarb-1500) was used to detect radioactivity.

Skin preparation

Dermatomed skin (approximately 600 μm thick) was obtained from local hospital sources. The tissue was kept frozen at −20°C and used within a week. To avoid any inter-individual differences, skin from only one donor was used. Before the experiment, 2-cm^2 large pieces of skin were excised and washed in MEM Eagle's with Earle's BSS (UCSF Cell Culture Facility) at room temperature. These tissue samples were then immediately mounted on the diffusion cells.

Permeation studies

Vertical, Marzulli-Bronaugh type flow through diffusion cells (Laboratory Glass Apparatus Inc., CA, USA) consisting of two water-jacketed cylindrical half cells, each having a volume of 2.8 ml and a diameter of 1 cm^2, were used. 1.5 cm^2 skin was mounted on the diffusion cells. The temperature of the diffusion cell was kept at 37°C by a circulating water jacket, which in turn maintained a temperature of 32°C on the skin (~skin temperature in vivo). The fluid in the receptor cells was constantly stirred by teflon-coated magnetic bars at about 200 rpm. The receiver compartment was continuously pumped with PBS (pH 7.4) at a steady flow rate of 2 ml/h. For in vitro methodology the guidelines proposed by Skelly et al. were followed (10).

Treatment

For topical application laurocapram has been applied in different organic solvents. Laurocapram is nearly immiscible in water. Water has been used as a co-solvent in topical formulations with laurocapram (1). Organic solvents may alter skin barrier function and cause irritation. This study observed the effect of laurocapram on penetration of the model irritant SLS. Therefore, a suspension of laurocapram in deionized water was prepared by mixing the solutions in a vortex mixer for about 15 min. The suspension was then immediately used for pretreating the skin.

After mounting the skin on the glass cells, the following pretreatments were performed:
(1) 2.5% laurocapram (100 μl), (2) 5% laurocapram (100 μl), (3)

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10% laurocapram (100 µl), (4) 99.3% laurocapram (100 µl), (5) 1% SLS (100 µl), (6) 100 µl deionized water (as a positive control) or (7) without treatment (as a negative control). The time of pre-exposure was 3 h.

After the pretreatment episode, excess laurocapram/SLS/water was carefully washed several times with deionized water to remove any residual left on the skin surface. The skin was also swabbed gently with soft tissue paper to ensure maximum removal of the applied solution. Thereafter, 100 µl of 1% [^{35}S]-SLS (1µCi/cell) was applied to the donor compartment, and each cell was covered (but not occluded) partially with aluminum foil to avoid evaporation of the donor fluid. The samples were then collected on a fraction collector over a period of 48 h.

Calculations

The cumulative amount of solute penetrating into the receptor compartment was plotted against time. The steady state flux was estimated from the slope of the linear portion of the cumulative time profile. Statistical comparisons were made using the paired Student's *t*-test. The level of significance was taken as $p=0.05$.

RESULTS

Fig. 1a–c shows the permeation curves of SLS after various treatments. The flux values of SLS after these various treatments are given in Table I. Pre-exposure of the skin to various increasing concentrations of laurocapram (2.5%, 5%, 10% w/v) indicated a linear increase in the flux values of SLS. However, 99.3% (undiluted) laurocapram treatment had no significant concentration-dependent effect on the permeation of SLS, and the flux values were comparable to the values obtained between 2.5%-5% laurocapram treatment (Table I).

The flux values were significantly higher for 5%, 10% and pure azone pretreatments, compared to water-treated controls (Table I). 2.5% laurocapram treatment, however, did not show a marked difference in SLS permeation compared to the water-treated control tissue ($p=0.19$).

Water is known to be a common irritant (11). A significant increase in the flux of SLS after pretreatments with water ($p=0.05$) and different concentrations of laurocapram was noted, compared to the untreated controls (no treatment) ($p<0.05$). Our studies observed that hydration of skin with water can induce an increase in the permeation of SLS ($p=0.05$). Permeation of SLS after pre-exposure to water was observed to be higher in the first 3 h, after which the values became relatively uniform throughout the experiment (Fig. 1a–c), whereas the permeation of SLS after laurocapram (Fig. 1b, c) and SLS treatment (Fig. 1a) steadily increased over time.

Furthermore, skin pretreatment with laurocapram had a more pronounced effect on SLS permeation (5% laurocapram, $p=0.01$; 10% laurocapram, $p=0.02$; pure laurocapram, $p=0.0001$), compared to a repeated application of SLS (Table I).

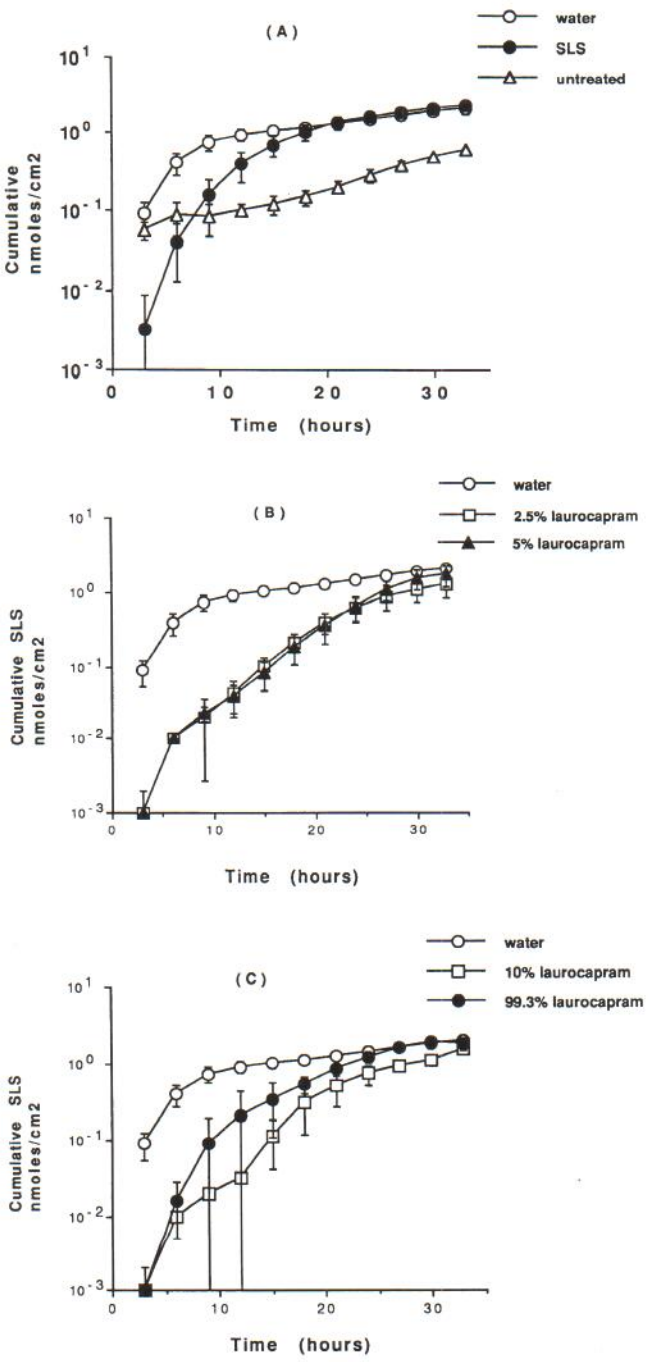

Fig. 1. Cumulative amounts of SLS penetrating excised human skin with time. Values expressed as mean±SD ($n=4$). (A) Significant permeation was observed for repeated SLS treatment. (B) Only 5% laurocapram treatment showed significant flux values compared to 2.5% laurocapram and water-treated samples. (C) Ten per cent and pure laurocapram treatment showed significant flux values compared to water-treated samples.

DISCUSSION

SLS may cause skin irritation above the CMC level (0.24%) (12). The events that occur after repeated topical exposure to SLS range from dryness, erythema, scaliness, and sometimes edema (13). Laurocapram has been reported to be non-irritating to human skin when applied at concentrations up to 50%, while pure laurocapram was only slightly more irritating than its mineral oil control (1). The enhancing effect of laurocapram is believed to be due to an increase in the fluidity of the intercellular bilayers of the stratum corneum without causing lipid depletion. Additionally, it increases the hydration of the stratum (5,6) by probably interacting with the fluid chains in the stratum corneum giving rise to various kinds of water- and/or oil-continuous lipid/water structures (7), thus enhancing penetration of various compounds through skin. SLS alters the cutaneous barrier function by direct interaction with keratin proteins, causing tightness and roughness of the

Table I. *Flux of ^{35}SLS after various treatments*
NS = not significant.

Treatment	Flux[a] nmoles/cm^2	P value*
Water	0.19 ± 0.05	
Untreated	0.11 ± 0.02	0.05
SLS	0.19 ± 0.00	NS
Laurocapram 2.5%	0.25 ± 0.05	NS
Laurocapram 5%	0.41 ± 0.12	0.02
Laurocapram 10%	0.45 ± 0.18	0.05
Laurocapram 99.3% (pure)	0.36 ± 0.02	0.006

* Values compared to water-treated controls.
[a] Values expressed as mean ± SD (n = 4).

skin (3). Increase in TEWL at the SLS-exposed site causes skin dehydration, which is also attributable to its action on intercellular lipids (4).

Concentration-dependent effects of laurocapram on the degree of SLS permeation were not observed above 10% in this study. Since laurocapram is reported to be most efficacious between 1–10% (w/v) in topical drug delivery (1,2,14), different concentrations of laurocapram were selected in this range (2.5%, 5%, 10% w/v) to study its effect on the permeation of SLS. Further investigations to determine if there are any concentration-dependent effects on the permeation of SLS between 10% and pure laurocapram are required.

Comparison of flux values of laurocapram-treated skin with water-treated controls suggests that the chances of skin irritation after exposure to SLS at the laurocapram-pretreated site may be of significance only above the concentration of 2.5%.

Water-hydrating effects on the skin have been well documented (11,15). Water, being dipolar in nature, can form bonds with many side-chains of keratin and can cause internal reorganization of hydrophobic groups. It can thereby have a major impact on the physical properties of keratin (16) and in turn on the skin barrier properties. Hydration of skin after pretreatment with water may thus increase the flux of SLS compared to untreated controls. A steep increase in the permeation of SLS in the first 3 h of the experiment may also be attributed to the obvious hydration of skin.

SLS is extensively used in various topical consumer products (1). Laurocapram, on the other hand, is used as a percutaneous penetration enhancer for a variety of topically applied drugs (1,10,17,18). An inadvertent exposure to SLS after topical treatment with formulations containing laurocapram is therefore possible. Although laurocapram is reported to cause no skin irritation in concentrations generally used (1), the above in vitro results indicate that pretreatment of the skin with laurocapram (above 2.5%) can lead to a significant increase in the permeation of SLS, which may result into an aggravated skin irritation at the laurocapram-preexposed site. SLS penetrates even into the deeper tissues under the site of application (muscle and deep muscle) and is known to cause tissue toxicity after a standard 24-h SLS treatment under occlusion (19). These results may therefore suggest a more deep tissue toxicity and an increased skin irritation due to SLS at any site, preexposed not only to laurocapram but possibly also to various other penetration enhancers.

Comparison of SLS flux after laurocapram and repeated SLS treatments suggests that the former could be a more effective permeation enhancer compared to SLS. After preexposure of the skin to SLS, the secondary and tertiary structures of the keratin proteins break down, thus creating new binding sites for the irritant upon repeated application (20). Furthermore, SLS fluidizes the lipid bilayers in the stratum corneum and inserts in between the lipid sheets (4,21,22). Repeated SLS treatment thus leads to accumulation of SLS in the epidermis (8 times higher) compared to a single 24-h treatment (19). The accumulation of SLS in the epidermis may therefore result in the retarded permeation of SLS through the skin, compared to the laurocapram-treated counterparts.

From the above study we therefore suggest that treatment of the skin with laurocapram possibly alters the barrier of the skin in such a way that it enhances the penetration of certain compounds better than SLS-pretreated counterparts. The effect of laurocapram on full thickness human skin after a single application was reported to persist for at least 120 h in vitro (23), but the reversibility of action appeared to be faster in vivo (24,25). Although the aim of the present study was only to observe the influence of laurocapram pretreatment on SLS permeation, more studies are needed to ascertain if this phenomenon is also true for other penetration enhancers. Taken together, the observations of increased SLS flux, elevating the chances of skin irritancy at laurocapram-pretreated sites, may be useful to screen other cutaneous enhancers for their clinical implications of causing secondary skin irritation. To ascertain the clinical importance of the in vitro results, further in vivo studies also need to be performed.

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