Sodium Lauryl Sulphate/ Ultrasound Combination for Transcutaneous Vaccine Delivery: Effect of changing Sodium Lauryl Sulphate Concentration on Antigen Permeation, Cavitation and Skin Damage

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Abstract Summary:

Low frequency ultrasound in combination with Sodium Lauryl Sulphate (SLS), a known transdermal chemical enhancer has been found to enhance delivery of molecules through the skin. In this abstract, we report the effects of changing SLS concentration on cavitation, protein permeation through the skin and skin damage.

Introduction:

Low-frequency ultrasound (US), applied to skin via a liquid coupling medium, has been shown to enhance the permeation of various molecules such as drugs and proteins across and into the skin[1]. The main mechanism of enhanced permeation is thought to be cavitation – formation and collapse of gas bubbles in the coupling medium. Violent collapse of gaseous bubbles produces shockwaves on the skin surface. It has also been shown that chemical enhancers such as sodium lauryl sulphate (SLS) can act in synergy with low-frequency US to further increase drug permeation into the skin[2, 3]. Tezel *et al*[3] suggested the synergy by US to be a result of increased dispersion to be a result of increased dispersion of the chemical enhancer in the skin. Later, Lavon *et al*[4] showed that US (which is accompanied by acidic by-products of sonolysis[5]) modifies the pH profile of the stratum corneum and suggested that this changes the ionization hence lipophilicity of SLS which resulted in increased partitioning and activity of SLS into the skin and hence increased drug permeation.

Interestingly, SLS in combination with US has been found to enhance skin permeation despite the fact that cavitation was reduced when SLS was present at 1%w/v[6]. The cavitation threshold (power needed to cause cavitation in a liquid) is known to be inversely proportional to the surface tension of the liquid[7] and the lack of cavitation in a 1%w/v SLS solution correlates therefore in agreement with the literature. The lack of cavitation that when SLS is present at 1%w/v shows that, processes other than cavitation are responsible for the US-assisted enhanced transdermal drug permeation.

In most of the studies reported in the literature, SLS has been used at 1%w/v. Lavon *et al*[4] rationalize this concentration as the one that is widely used in cosmetic and pharmaceutical products and is FDA-approved for topical preparation. However, SLS is a known skin irritant and its irritancy may be increased when used concurrently with US. It is also possible that 1%w/v SLS may not be the optimal level for maximum permeability coupled with minimum damage to skin.

Our aim was therefore to determine the optimal protocol for maximum vaccine permeation with minimum skin irritation and we investigated the effect of lowering concentration in the coupling medium on drug permeation and on skin toxicity. In vitro experiments using rat skin were conducted to test permeation of a radiolabelled protein (model vaccine). Other in vitro experiments using aluminium foil were conducted to quantify the number of pits (an indication of cavitation) caused by the experimental protocols. While in vivo studies in rats were conducted to test skin tolerability of the experimental conditions.

Experimental Methods

The effect of increasing SLS concentration on protein permeation into rat skin in vitro

Permeation studies using vertical Franz cells were conducted where full-thickness rat skin was used as the membrane. The donor compartment was filled with 20 ml of coupling medium (water or SLS aqueous solution at different %w/v concentrations: 0.001 /0.004 /0.01/ 0.04 /0.1 /1) and US (30% amplitude, 0.5s on, 0.5s off pulse wave for a total sonication time of 2 min) was applied. Following US application, the coupling medium was removed, the skin was rinsed and blotted dry, and 50µl of Iodine-125 labelled bovine serum albumin (BSA, a model antigen) was applied onto the skin. After 24h, the levels of radioactivity (indicating protein levels) in the receptor compartment and were measured.

The effect of increasing SLS concentration on the pitting on aluminium foil

Pitting (the formation of pits) on aluminium foil has been used as an indication of cavitation that can be caused by US-induced cavitation[6]. In our studies, we measured the number of pits formed on aluminium foil and determined correlations, if any, between the number of pits, protein permeation in vitro and skin irritation in vivo. The experiments were conducted as described above except for the fact that aluminium foil was used as the membrane and the number of pits formed on the aluminium surface was visually counted after sonication.

In vivo experiments to determine tolerability of SLS/Ultrasound combinations

The abdominal skin of rats was carefully shaven 24h before the experiment. Anaesthetized animals laid on their back and a custom-made flanged cylinder was attached to the skin. The chamber was filled with a coupling medium (water or SLS aqueous solution) and US (same protocol as for the in vitro studies) was applied. After sonication, the skin was washed; blotted dry and erythema, blood flow and Transepidermal Water Loss (TEWL) were measured respectively at 5, 15, 30, 45 and 60 min post sonication. The animal was killed and the sonicated skin was prepared for histological analysis.

Results and Discussion:

The surface tension of the different coupling media and the effects of increasing SLS concentration in the coupling medium on: (i) protein permeation in vitro, and (ii) number of pits formed on aluminium foil are shown in Figure 1.

We can see that: (i) as expected, increasing SLS concentration resulted in a marked decrease in surface tension when SLS concentration exceeded 0.01 %w/v. After this concentration, sufficient numbers of SLS molecules were present at the liquid-air interface to cause a reduction in surface tension.

(ii) Increasing SLS concentration (& lowering surface tension) in the coupling medium caused a decrease in pitting. During cavitation, some of the gas bubbles are expected to hit the aluminium foil membrane and thereby cause pits on the surface. While aluminium foil is a very different matter to animal skin, cavitation is a property of the liquid and therefore pitting on aluminium foil can be used as an indication of the likelihood of gas bubbles being formed, hitting the skin and thereby increasing drug or vaccine permeation into and through the skin. The decrease in pitting on aluminium foil indicating reduced cavitation with decreasing surface tension correlates with the literature.[3, 7]

SLS at concentration less than 0.004%w/v seems to be too low to affect permeation. Surprisingly, increasing SLS concentration from 0.004 %w/v to 0.04 %w/v resulted in a marked decrease in protein permeation, reflecting the decreasing pitting on aluminium foil. A further increase in SLS concentration from 0.04 %w/v to 1 %w/v resulted in increased protein permeation, despite the absence of pitting. The partial correlation between the pitting and permeation profiles suggest that at lower SLS concentration, cavitation (reflected in pitting) remains the main mechanism by which protein permeates into the skin. At higher concentrations (>0.04%w/v), however, other mechanisms of surfactant-induced permeation enhancement such as swelling of the stratum corneum and interaction with intercellular keratin and extraction of lipids from stratum corneum, enhanced by US, become important.

Surprisingly, lower concentrations of SLS (0-0.004%w/v) resulted in similar permeation enhancement compared to 1%w/v SLS when combined with US. This is contrary to the literature, where addition of SLS at 1%w/v has been shown to enhance drug permeation[2, 3]. A direct comparison of our results with the literature may not be applicable as different US protocols and different permeants were used. In our case, a large permeant, a larger volume of coupling medium was used; a shorter probe distance and a longer pulse length were used. These parameters have been previously optimized and thus, in absence of SLS, sufficient US power was generated such that addition of SLS did not cause an observable increase in permeation. In addition when 1%w/v SLS was used on its own, very little protein permeated through the skin (Shown on Fig 1). No significant enhancement was therefore achieved.

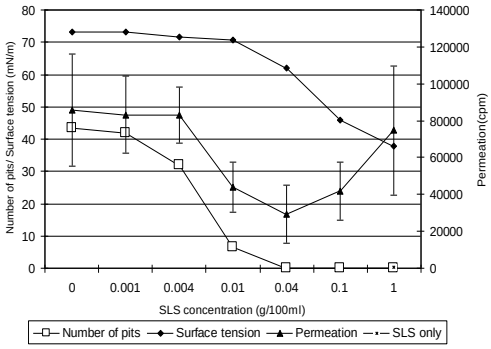

Fig 1 Effects of the surface concentration of coupling media on surface tension (n=8), protein permeation into the receptor phase and aluminium foil pitting (n=5).

In vivo investigation showed increased erythema scores with increasing SLS concentration and histological analyses showed greater skin damage with higher SLS concentrations. LDV and TEWL however, did not show significant difference between the different coupling media. This raises issues about correlating TEWL and LDV measurements with skin damage.

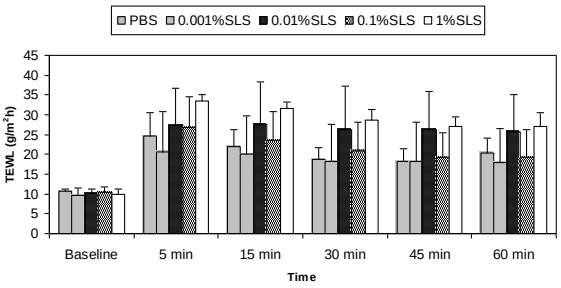

Fig 2 Effects of the surfactant concentration of coupling media on TEWL (n=5)

Conclusions:

Our results confirmed previous reports on reduced cavitation when SLS is included in the coupling medium. Our results also indicate that lower concentrations of SLS may be as effective as high concentration, when combined with optimized US parameters to increase permeation of large molecules. Lower US concentrations/US combinations were found to cause less damage to the skin.

References:

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