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# Influence of lidocaine hydrochloride and penetration enhancers on the barrier function of human skin

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ABSTRACT

Skin penetration enhancers (SPEs) are commonly employed in pharmaceutical and personal care products. These compounds transiently alter the barrier properties of the skin and we have previously investigated the effects of specific SPEs on skin barrier function *in vivo*. In the present study the effects of incorporation of an active pharmaceutical ingredient (API), lidocaine hydrochloride (LID HCl) in the SPEs previously studied were investigated. Solutions of LID HCl were prepared and applied to the volar forearm of human subjects with occlusion for 24 h. Subsequently, tape stripping and *trans* epidermal water loss (TEWL) measurements were conducted for treated and control sites. The activities of the desquamatory proteases, kallikrein 5 (KLK 5) and kallikrein 7 (KLK 7) and API content were also measured from the tape strips. The propylene glycol (PG) formulation increased TEWL significantly ($p < 0.05$) compared with the other SPEs and a mixture of the SPEs. However, only the isopropyl myristate (IPM) solution altered protease activity with a significant observed increase in kallikrein 5 (KLK 5). Incorporation of LID HCl appeared to ameliorate the effects of some of the SPEs on TEWL measurements compared with our previous study. Overall uptake of LID HCl into skin from the various formulations correlated very well with changes in TEWL. The findings should have implications for the choice of SPEs in topical and transdermal formulations, particularly where the skin barrier function of patients is already impaired for example in atopic eczema or psoriasis.



## 1. Introduction

Skin penetration enhancers are found in a wide range of pharmaceutical and cosmetic products. Their primary function is to enhance the delivery of actives to and through the skin. They achieve this *via* transient modulation of skin barrier function by a number of mechanisms; alteration of the solubility of a molecule in the stratum corneum (SC) or enhancement of the diffusion of the molecule through the SC because of perturbation of the lipid pathway, or both (Lane, 2013). We hypothesised that these transient effects should result in changes, at the molecular level, of the SC. Subsequently we reported that propylene glycol (PG) elevates the activity of the desquamatory protease, kallikrein 7 (KLK 7) as well as *trans* epidermal water loss (TEWL) when applied under occluded conditions for 24 h to the volar forearm of human subjects (Mohammed et al., 2014).

In the present work we have extended our previous study to examine the effects of simple formulations of a model active pharmaceutical ingredient (API), lidocaine hydrochloride (LID HCl), on TEWL and protease activity. This API is used as the base or hydrochloride salt (Fig. 1) in a range of topical formulation including gels, patches and solutions; the therapeutic applications of the preparations are provision of surface anaesthesia or management of post herpetic neuralgia (PHN). Simple solutions of LID HCl in isopropyl myristate (IPM), PG, propylene glycol laurate (PGL), Transcutol$^{TM}$ (TC) as well as a mixture of the four vehicles were prepared. These solvents were selected as they were investigated in our previous study and they are commonly used in both topical and transdermal preparations. A mixture of the solvents was also selected for evaluation because SPEs may act synergistically (Lane, 2013). As TEWL and drug uptake into skin should also provide insight into the interaction of the formulations with skin these parameters were also examined.

## 2. Materials and methods

### 2.1. Materials

LID HCl, IPM and PG were purchased from Sigma–Aldrich, UK. PGL and TC were a kind gift from Gattefossé (St. Priest, France).

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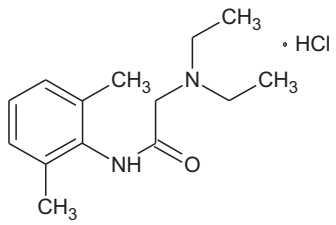


**Fig. 1.** Lidocaine hydrochloride.

Solutions of LID HCl were prepared at 5% w/v in PG, PGL and TC. LID HCl was used at its saturated solubility in IPM and in the IPM:PG:PGL:TC mixture as the respective saturated solubility values were <5% w/v in these vehicles. D-Squame™ tapes were obtained from CuDerm Corporation (Dallas, TX, USA).

### 2.2. Methods

#### 2.2.1. Volunteer recruitment and application of formulations

Ethical approval (REC reference number: 09/H0722/14) was obtained from the Camden & Islington Research Ethics Committee. Six healthy volunteers, who gave informed consent, were randomly assigned six application sites on the left and right volar forearms (three sites on each arm). At each delineated site, TEWL measurement was conducted with an Aquaflux™ AF103 (Biox Ltd., London, UK) to measure baseline values before applying any formulation. 76 μL of the formulation was applied by micropipette onto the skin over a 3.8 $cm^2$ surface area (20 μL/$cm^2$). After application, a piece of filter paper with an area of 3.8 $cm^2$ was placed on the skin and then left for 5 min and then fixed to the area with surgical tape. Control sites, with no formulation, were covered in the same manner with filter paper and occlusion with surgical tape. Volunteers were asked not to disturb the area of application but were allowed to shower and bathe as normal. After 24 h application, the surgical tapes and filter paper were gently removed. The application sites were left undisturbed for 20 min prior to TEWL measurement to avoid any influence of hydration of the skin by occlusion. After measurement of TEWL, tape stripping was conducted 10 times with Standard D-Squame® discs with an area of 3.8 $cm^2$. During the sequential tape stripping TEWL values were also measured after the 4th, 7th and 10th tape stripping to investigate any increase in TEWL (Mohammed et al., 2014).

#### 2.2.2. Measurement of protein content, protease activity and LID HCL

The protein content removed from 10 tape strips was quantified by absorption measurements at 850 nm for each tape with a Squame Scan™ 850A (Heiland Electronic, Germany) which has been custom designed for the assay of protein content in standard D-Squame discs. For protein quantification the following equation was used (Voegeli et al., 2007):

$$C_{\text{protein}}\left(\frac{\mu g}{\text{cm}^2}\right) = 1.366 \times \text{absorption}(\%) - 1.557 \quad (1)$$

After the protein content measurement, tapes were transferred into 2 mL Eppendorf tubes and extracted in 750 μL of Tris–HCl buffer at pH 8.0 containing 0.5% Triton X-100. This extract was used for analysis of LID HCl in the SC and desquamatory protease activity as reported previously (Mohammed et al., 2011a, 2014). Analysis of KLK 5 and KLK 7 activity was conducted on pooled tape strippings (2–4, 5–7, 8–10). Protease activity was measured by quantifying the amount of amino methylcoumarin released from fluorogenic peptide substrates with HPLC (Mohammed et al., 2011a).

Analysis of LID HCl in the SC was performed for tape strips (2–10). The API was extracted as described above with vortexing at ambient temperature for 15 min. The extract was then diluted with ethanol and centrifuged at 10,000 rpm/min for 5 min (Eppendorf Centrifuge 5415D, Eppendorf AG, Germany). Samples were analysed by HPLC using a Discovery® HS $C_{18}$ (15 cm × 4.6 mm i.d., 5 μm, Supelco, UK) column and a $C_{18}$ security guard™ cartridge (4 × 2.0 mm i.d., 5 μm, Phenomenex, UK). The mobile phase consisted of methanol:water:trifluoroacetic acid (30:70:0.1, v/v/v). Detection was achieved with a UV detector at a wavelength of 215 nm. The flow rate of the mobile phase through the column was 1 mL/min and the injection volume used was 20 μL. The retention time was approximately 7 min. Calibration curves were constructed and the HPLC method was validated with specificity, linearity, accuracy and precision being determined; the lower limit of quantification was 0.5 μg/mL.

#### 2.2.3. Statistical analysis

Data were checked for normality of distribution using the Kolmogorov–Smirnov test. Statistically significant differences were determined using one-way analysis of variance (ANOVA), and comparison of means for different groups was performed using a *t*-test (SPSS version 18, United States). For all analyses a probability of $p < 0.05$ was considered statistically significant.

## 3. Results and discussion

### 3.1. TEWL measurement

The difference in TEWL values before and after 24 h application of the selected 5 formulations and control is shown in Fig. 2a.

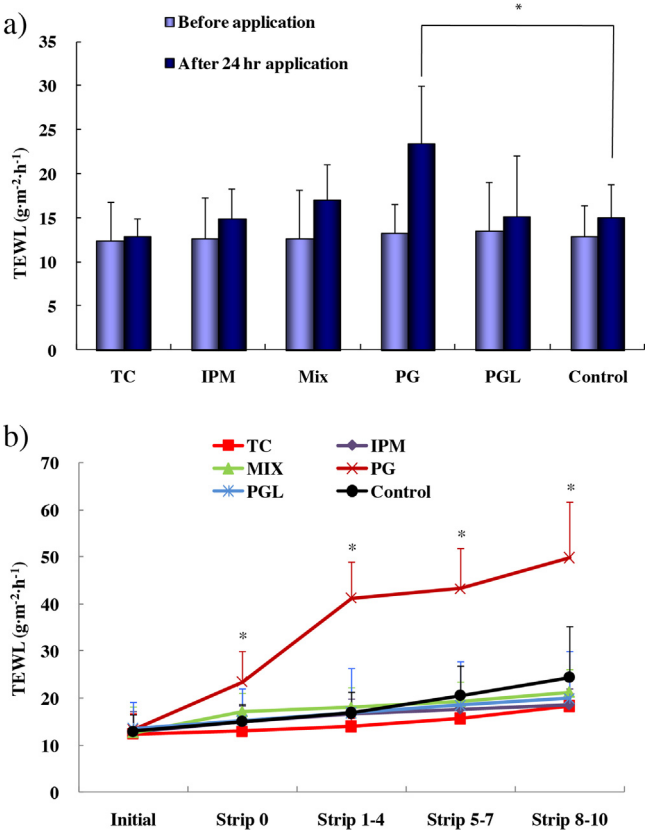


**Fig. 2.** (a) Change in TEWL value before and after 24 h application of the selected formulations to the volar forearm of 6 healthy volunteers (Mean ± S.E.M., $n = 6$). (b) Change of TEWL value during tape stripping of the volar forearm of 6 healthy volunteers following application of LID HCl formulations for 24 h under occlusion (Mean ± S.E.M., $n = 6$).