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Influence of skin penetration enhancers on skin barrier function and skin protease activity

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ABSTRACT

In order to overcome the skin's excellent barrier function formulation scientists often employ skin penetration enhancers (SPEs) in topical and transdermal formulations. The effects of these compounds on skin health is still not well understood at the molecular level. The aim of the present work was to probe the effects of some common SPEs on desquamatory protease activity in healthy skin. The SPEs studied were isopropyl myristate (IPM), propylene glycol, (PG), propylene glycol laurate (PGL) and Transcutol™ (TC). Occluded infinite doses of each SPE were applied to human volunteers for 24 h. Transepidermal water loss (TEWL) measurements were taken before and after application of SPEs. Tape strips were collected from the treated sites to determine protein content and the activity of two desquamatory proteases kallikrein 5 (KLK5) and kallikrein 7 (KLK7). TEWL values were also measured after tape stripping. PG was found to elevate both TEWL values and KLK7 activity to a significant extent ($p < 0.05$). No significant effects were observed for the other SPEs. The ability of PG to alter the skin barrier at the macroscopic level and the influence of the molecule on protease activity reported here may have implications for its use in topical formulations used for the management of impaired skin barrier function such as atopic eczema or psoriasis.

1. Introduction

Skin penetration enhancers (SPEs) are used in many topical and transdermal medicines to promote the transport of drugs into and across human skin (Lane, 2013). The proposed mechanisms of action of chemical enhancers may be categorized into two groups. The first mechanism is based on an increase in the solubility of the permeant in the stratum corneum (SC) by transiently changing the solubility parameter of the skin. Consequently, there may be an improvement in the flux of the drug through the skin (Hadgraft, 2004; Watkinson et al., 2009; Lane, 2013). Simple solvents, such as propylene glycol, ethanol, and Transcutol®, are believed to act in this way (Lane, 2013). The second mechanism involves a change in the order of the structured lipids of the SC. This effectively fluidises the lipid domain thereby increasing the diffusion coefficient of the permeant. It is considered that IPM acts in this way that is, it enters the lipid layers of the SC and reduces the close packing of fatty acids within the skin (Lane, 2013). This ultimately results in increased mobility of SC lipids and reduced diffusional resistance to permeation.

Increasing permeant transport by altering the SC ultrastructure inevitably means a change in the skin barrier properties. A range of techniques have been used to characterise and quantify such changes. These include non-invasive methods such as measurement of Transepidermal water loss (TEWL) or capacitance (Angelova-Fischer et al., 2010; Mohammed et al., 2011a,b) as well as more invasive methods including tape stripping (Di Nardo et al., 1998) or skin biopsy (Lee et al., 2012). Recently, the levels of protease activity and TEWL values have been reported to be lower for the SC of protected skin sites compared with values of the same measurements for skin sites that are exposed to the environment (Voegeli et al., 2007a). In a further paper the same authors reported increased serine protease activities in the SC of patients with acute eczematous atopic dermatitis compared with healthy subjects (Voegeli et al., 2009). These elevated protease activities were associated with impaired barrier function, irritation, and reduced skin capacitance.

The aim of the present study was to investigate the effects of some common SPEs on skin barrier function. The SPEs selected for examination are commonly used in topical and transdermal formulations and included isopropyl myristate (IPM), Transcutol™ (TC), propylene glycol (PG) and propylene glycol laurate (PGL). TEWL measurement, protein content quantification and protease activity assessment were used to determine any effects of the SPEs on the skin of healthy human subjects. Tape stripping was con-

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ducted to collect the SC for measurement of protein content and serine protease activity. The proteases selected for evaluation were members of the kallikrein (KLK) family, namely KLK5 and KLK7. KLK5, also known as SC tryptic enzyme (SCTE) and KLK7, also known as SC chymotryptic enzyme (SCCE) have been reported to be related to SC turnover and desquamation in the epidermis (Egelrud and Lundstrom, 1991; Lundstrom and Egelrud, 1991; Brattsand and Egelrud, 1999). These two enzymes are implicated in desquamation by digestion of (corneo) desmosomes and inhibition by desquamation-related serine protease inhibitors (Borgono et al., 2007).

2. Materials and methods

2.1. Subjects and study protocol

Ethical approval (REC reference number: 09/H0722/14) was obtained from the Camden & Islington Research Ethics Committee, London, U.K. Six healthy volunteers who gave informed consent were randomly assigned 5 application sites on the left and right volar forearms (2 or 3 sites on each volar forearm). 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, left for 5 min and then fixed to the area with surgical tape (Boots, U.K.). For the control site no formulation was applied and filter paper was taped to the site as for the treated sites.

After a 24 h application period, the surgical tapes and filter paper were gently removed. The application sites were left undisturbed for 20 min prior to TEWL measurement to limit any influence of hydration of the skin by occlusion. After measurement of TEWL, tape stripping was conducted 10 times with Standard D-Squame® discs (Cuderm Corp, TX, USA) with an area of 3.8 cm^2. TEWL values were also measured following removal of tapes 4, 7 and 10.

2.2. Measurement of protein content and protease activity

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., 2007b):

$$C_{protein}\ (\mu g/cm^2) = 1.366 \times \text{Absorption}\ (\%) - 1.5857.2$$

After the protein content measurement, 2–10 tapes were transferred into a 2 mL Eppendorf tube and extracted with Tris HCl buffer as reported previously (Mohammed et al., 2011b). Analysis of the proteases was conducted on pooled tape strippings (2–4, 5–7, 8–10) by quantifying the amount of aminomethyl coumarin (AMC) released from fluorogenic peptide substrates with HPLC (Mohammed et al., 2011b).

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), where the comparison of the means within the different groups was performed using a *t*-test (Microsoft Excel 2010, WA, U.S.). For all analyses a probability of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. TEWL measurements

The difference in TEWL values before and after 24 h application of the selected SPEs to specific sites and the control site is shown in Fig. 1a. Occlusion with a filter paper and surgical tapes increased the TEWL values significantly for all groups including the control site ($p < 0.05$). However, PG and TC resulted in a greater TEWL increase values compared with the rest of the tested formulations ($p < 0.01$). TC is believed to act in a similar manner to PG as a penetration enhancer but there are no studies in the literature which have elucidated its exact mechanism of action. The changes in TEWL during tape stripping of all treated sites are shown in Fig. 1b.

Significantly different increases in TEWL were observed not only after the 24 h application of PG (Tape strip 0) but also for the tape stripping process (up to 10 tapes) when compared with the control, consistent with the data in Fig. 1a. TEWL is a macroscopic descriptor of skin barrier function and elevations in TEWL values are associated with reduced skin barrier function (Lodén et al., 1999; Watkinson et al., 2002). Grubauer et al. (1989) demonstrated that water transit may be a signal which stimulates epidermal lipid synthesis. These findings suggested that epidermal lipid synthesis increases as water loss rates are increased, which is associated with the recovery of SC barrier function. Increased TEWL after application of PG was previously demonstrated in porcine epidermis *in vitro* (Levang et al., 1999). PG is widely used as a vehicle for topical medicines, cosmetic lotions, and in shampoos, and is often added to cosmetics and formulations because it is a humectant (Rowe et al., 2009). However, there are few scientific papers which demonstrate the moisturizing ability of PG in human skin and some authors (Ostrenga et al., 1971; Goodman and Barry, 1989) have suggested that PG dehydrates the skin. Wide and small angle X-ray diffraction studies (Brinkmann and Muller-Goymann, 2005) revealed that PG molecules integrate into hydrophilic regions of the packed lipids and increase the distance in the lamellar phase by incorporation between the hydrophilic head groups of the bilayers and in the perpendicular direction to the bilayer. The above mechanism of PG function on the SC may explain the elevated TEWL after PG application.

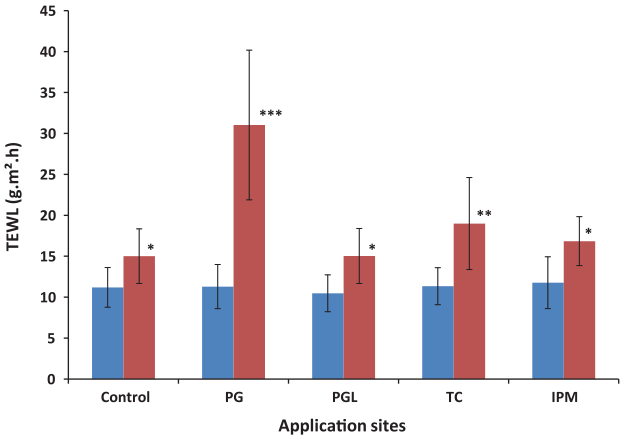

Fig. 1a. Change in TEWL value before (■) and after (■) 24 h application of the selected formulations to the volar forearm of 6 healthy volunteers. Each data point represents the mean ± S.D, $n = 6$ (before vs. after application; $^{*}p < 0.05$, $^{**}p < 0.003$, $^{***}p < 0.001$).