Impact of pharmaceutically-used emulsifiers on in-vitro and in-vivo skin barrier function by trans-epidermal water loss (TEWL), confocal Raman microscopy (CRS), and ceramide profiling by liquid chromatography-mass spectrometry (LC-MS)

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Emulsifiers are common excipients in dermal products that stabilize creams and emulsions. But emulsifiers can be skin irritative, so they should be tested regarding their tolerability before applying them onto the skin [1,2,3,4]. In the following, a systematic investigation was conducted using in-vitro porcine samples. These samples were measured with trans-epidermal water loss (TEWL), confocal Raman spectroscopy (CRS), and liquid chromatography-mass spectrometry (LC-MS) to analyze the impact of sorbitan esters (Span 40, 60, 80, and 120) as w/o-emulsifiers. Additionally, in-vivo human skin was studied with 12 healthy volunteers, six female, and six male participants. The measurements included TEWL and LC-MS analysis to study six non-ionic o/w- or w/o-emulsifiers (including PEG-fatty alcohol ethers and sorbitan esters). The in-vivo study was performed according to the declaration of Helsinki, approved by the ethics committee of the University clinics of Tuebingen (221/2022BO2), and informed written consent was obtained from the volunteers. Water was used as a negative control and sodium lauryl sulfate (SLS) as a positive, irritant control. TEWL measurement is a widely used method to determine the integrity of the skin barrier. It is also recommended by the European Medicines Agency (EMA) draft guideline on quality and equivalence of topical products [5] to monitoring skin integrity. CRS is another method to characterize impairment of the stratum corneum (SC) as the lipid content and thickness of the SC can be measured [2]. Decreasing lipid contents or thinner SCs indicate skin impairment. The impact of emulsifiers on TEWL and SC lipids was measured using the same skin samples. After TEWL measurement, the SC was isolated by a trypsin digestion protocol. Lipids content and conformation was measured by CRS. LC-MS was used afterwards to determine the ceramide profile [6,7]. To study the correlation between the different methods, TEWL changes were compared and linked to changes in each individual ceramide species using Partial Least Squares and Ridge Regression. The in-vitro

TEWL measurements showed that the sorbitan esters showed no significant changes compared to the water-treated sample. The lipid content, measured by CRS, was mostly decreased except for Span 120. Conformation, lateral packing order, and SC thickness, also measured by CRS, showed no significant differences compared to the water reference sample. EO-type ceramides showed a positive correlation with higher TEWL values, phytosphingosine-type ceramides and longer chained ceramides showed an inverse relationship with TEWL. In-vivo TEWL measurements showed that the applied emulsifiers induced no significant changes compared to the water-treated sample (negative control) while SLS did. Human long-chain ceramides have smaller regression coefficients, and short-chain ceramides have higher regression coefficients, the latter reflecting more severe skin damage. Different effects were found in phytosphingosine-based ceramides and sphingosine-based ceramides, non-EO hydroxy sphingosine-based ceramides, as well as ceramide NDS, and EOH. These detailed investigations help us to obtain a better understanding of the interactions of different emulsifiers of o/w and w/o type with skin in general.

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