The effect of ethanol on phospholipase A_2, lipase, β-glucocerebrosidase and sphingomyelinase activities in the human stratum corneum

The permeability barrier of the stratum corneum consists primarily of intercellular ceramides, fatty acids and cholesterol. These lipids originate from precursor lipids in the intercellular space. Lamellar bodies secrete precursor lipids and specific enzymes within the stratum granulosum/stratum corneum interface which convert lipids to their mature forms. Phospholipid, triacylglycerol, sphingomyelin, and glycosylceramide are processed by phopsholipase, lipase, sphingomyelinase, and β-glucocerebrosidase, respectively. Disease as well as environmental influences can alter the secretory behaviour of lamellar bodies, enzyme levels and hence the intercellular lipid composition. The aim of this work was to develop selective fluorescence-based assays to detect phospholipase A_2, lysosomal lipase, β-glucocerebrosidase and sphingomyelinase activities in human skin based on the tape stripping method and to use them to examine the skin barrier function following repeated topical ethanol application.

Analytical grade ethanol was applied to the ventral forearm ten times per day (40µl/cm^2) over 28 days. Transepidermal water loss measurement and tape stripping (30 tapes, D-Squame® tapes) was conducted initially and weekly for the consecutive five weeks. Protein recovered by the tapes was quantified by infrared absorbance with a densitometer. The tapes were analysed in triplicate and extracted in the appropriate reaction buffer (phospholipase A_2 and lysosomal lipase, Tris-HCl, pH 7.5; β-glucocerebrosidase, Na actetate, pH 5.6; sphingomyelinase, Na acetate, pH 4.7). The fluorogenic substrates PED6, 4-methylumbelliferyl oleate, 4-methylumbelliferyl-β-D-glucopyranoside, and 6-Hexadecanoylamino-4- methylumbelliferyl-phosphorylcholine were used to measure phospholipase A_2, lysosomal lipase, β-glucocerebrosidase and sphingomyelinase levels, respectively. Enzyme activities were recorded as fluorescence intensities of product formed measured after incubation at 37°C for 990, 990, 200, and 420 minutes, respectively.

Transepidermal water loss increased linearly with treatment time by a factor of 5.4 and returned to initial values after a one-week absence of any treatment. The overall protein removal over 30 tapes was decreased by 28.4% and shifted towards more protein removed by the outermost tapes compared to initial measurements. Enzyme activities were observed to be higher in the deeper stratum corneum layers. With increased duration of ethanol application enzyme activities increased, which was more distinct in the deeper layers (factor 4.7) than the outer skin layers (factor 3.7). After stopping application activities returned to initial values within one week for phospholipase A_2, lipase, and sphingomyelinase but not for β-glucocerebrosidase.

The assays developed in this study represent a non-invasive and selective method to measure four key enzymes involved in stratum corneum lipid processing. These methods allow deeper insights into the behaviour of lipid barrier maintenance and inflammatory response of the skin under ethanol treatment. This will also allow evaluation of the influence of further excipients and active ingredients in topical formulations on skin health.