ABSTRACT

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Title of Doctoral Thesis: Effect of amino acids derivatives and ceramides on skin barrier function

The main goal of my thesis was to contribute to the understanding of behavior of the ceramides and skin barrier function modulators based on amino acids derivatives, as well as to elucidate the relationship between their structure and activity.

Transdermal permeation enhancers are compounds that temporarily decrease skin barrier properties to promote drug flux. In the first part of my thesis, enhancers with amino acids (proline, sarcosine, alanine, β-alanine, and glycine) attached to hydrophobic chain(s) via a biodegradable ester link were investigated. The double-chain lipid-like substances displayed no enhancing effect, whereas single-chain substances significantly increased skin permeability. The most active enhancer - proline derivative L-Pro2 reached enhancement ratios of up to 40 at 1% concentration, which is higher than that of the well-established and standard enhancers Azone, DDAIP, DDAK, and Transkarbam 12. No stereoselectivity was observed. L-Pro2 acted synergistically with propylene glycol and infrared studies revealed that its mechanism of action includes fluidization of skin barrier lipids with no significant effect on stratum corneum proteins. L-Pro2 action was at least partially reversible as measured by skin electrical impedance. Toxicity in keratinocyte (HaCaT) and fibroblast (3T3) cell lines showed IC_{50} values ranging from tens to hundreds of μM, which is comparable with standard enhancers. *In vivo* transdermal absorption studies in rats confirmed the enhancing activity of L-Pro2 and showed its negligible skin toxicity and minimal effect on transepidermal water loss. Furthermore, L-Pro2 was rapidly decomposed in plazma. These properties make L-Pro2 a good candidate for potential clinical use.

Stratum corneum ceramides play an essential role in the barrier properties of skin. However, their structure–activity relationships are poorly understood. We have recently

shown that the acyl chain length in non-hydroxy acyl sphingosine-type (NS) ceramides is highly important for their barrier function and that its shortening decreased the skin diffusion resistance with maxima at ceramides with 4-6C chain length. In the second part of this thesis we investigated the effects of acyl chain length of NS type ceramides on the permeability and the thermotropic phase behavior of a model SC lipid membranes composed of ceramide, lignoceric acid, cholesterol and cholesterol sulfate to gain some basic understanding of the observed differences in permeability. Short-chain ceramides decreased membrane electrical impedance and increased the permeability of theophylline in the model SC membranes similar to their effects found in the skin. The thermotrophic phase behavior was studied by differential scanning calorimetry and infrared spectroscopy. Those ceramides that had the greatest impact on the membrane permeability broaden and downshift phase transitions of the model SC membranes. ATR-FTIR studies revealed that, at the skin temperature, all membranes showed high lipid chain conformational order and presence of orthorhombic chain packing together with very similar polar head region. Thermal phase behavior of membranes revealed that membranes with short-chain ceramides form separate domains rich either in ceramides or lignoceric acid. Moreover relative CH_2 intensity at 32°C was weaker in the most permeable membranes suggesting presence of ceramide-rich domains with lower lipid density. In conclusion, this work confirmed that the long hydrophobic chains in the NS-type ceramides are essential for maintaining the skin barrier function. Moreover, the model SC lipid membranes reconstituted on a filter support proved to be a valuable tool to study the relationships between the ceramide structure and SC membrane permeability as well as the underlying mechanisms on a molecular level.

In the last part of my thesis a number of new homologs of pseudoceramide 14S24 were synthesized to study their barrier-repair ability and to evaluate the influence of the length of hydrophobic chains on their activity. This work included evaluation of two different methods for barrier pertubation and study of various donor vehicles. However, in this part of the work we were unable to assess the activity of the prepared compounds, because considerable variation in the permeability of the skin was observed. The possibility to avoid observed variability is the study of these homologs by model SC lipid membranes, which were developed in the second part of the thesis. Nevetheles, methods and results presented in this section may be helpful in further studies of this issue.