ABSTRACT

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Title of Doctoral Thesis **Study of Effect of Ceramide Hydroxylation on Permeability and Microstructure of Model Lipid Membranes**

Ceramides (Cer) occur in intracellular spaces of the stratum corneum, the outermost mammalian epidermal layer, where they along with other lipids (free fatty acids, cholesterol) form a skin lipid barrier.
Besides sphingosine- (Cer NS) and dihydrosphingosine-based Cer (Cer NdS), in the healthy mammalian epidermis some polar Cer are present, i.e., Cer based on phytosphingosine (with hydroxyl group in position 4, i.e., phytoceramides) and Cer based on 6-hydroxysphingosine (with hydroxyl group in position 6, i.e., 6-hydroxyceramides). However, the role of phytoceramides (Cer NP) and 6 hydroxyceramides (Cer NH) in the skin barrier function has not been clarified. Moreover, 6 hydroxyceramides are not commercially available. Therefore, the first aim of this work was to synthesize physiological 6-hydroxyceramides. The synthesis of 6-hydroxylated Cer, i.e., Cer NH with long (C24:0) and Cer EOH with ultralong acyl with ester-linked linoleic acid is reported. The triple bond was reduced into trans-double bond by using selective applied Trost hydrosilylation-protodesilylation catalyzed by [Cp*Ru(CH3CN)3]PF6 in dry acetone.
The diversity of Cer in the skin and the reason why epidermis synthesizes phytoceramides, are still unknown. The aim of this thesis was to investigate the effect of C-4 hydroxylation of Cer on barrier properties and phase behaviour of phytoceramides in model membranes. Model membranes were prepared as a molar mixture of Cer NP, or Cer NdS, or Cer NS, lignoceric acid, and cholesterol with an addition of cholesteryl sulfate. Models were investigated by permeation and biophysical (infrared spectroscopy and X-ray powder diffraction) experiments. Hydroxylation of Cer in position 4 leads to less ordered lipids in models, lipids are phase separated and model Cer NP membranes are more permeable than those based on Cer NS or Cer NdS. Using deuterated lignoceric acid in model membranes we found out that hydroxylation in position 4 in phytoceramides restricts the miscibility of lipids with deuterated fatty acid. From this study, we can conclude that phytoceramides have not only barrier function in epidermis but they probably play a role in other processes.
The uniqueness of 6-hydroxyceramides in mammalian epidermis has not been clarified. Therefore, the aim of this work was to study Cer based on 6-hydroxysphingosine (Cer NH) in model membranes. Multilayer lipid membranes were prepared in following composition: Cer NH/mixture of free fatty acids (C16-C24)/cholesterol/cholesteryl sulfate. Hydroxylation of Cer in position 6 increases the model membrane permeability to water and lipophilic molecules, but also increases the opposition to electrical current. Moreover, the 6-hydroxylation prevents the good mixing of Cer NH with deuterated fatty acids. Using the investigation of model membrane microstructure, we found out that Cer NH probably facilitates the formation of lamellar phase with unusual long periodicity. Long periodicity phase is essential for the correct barrier function. This fact could explain the unique role of 6-hydroxyceramides in the skin.
To elucidate the role of physiological D-erythro configuration in sphingosine and dihydrosphingosine Cer, the aim of this work was to synthesize non-physiological L threo (dihydro)ceramides. From the investigation of barrier properties and phase behaviour of L threo Cer NS and L-threo-Cer NdS in model membranes, we found out that the stereochemistry conversion in Cer polar head leads to increase of cohesive forces of lipids in model membranes. While L threo configuration has no effect on lamellar lipid organization, the change of Cer stereochemistry leads to decrease of miscibility of deuterated fatty acids with L-threo-Cer NS or L-threo-Cer NdS and to increase of permeability of model lipid membranes as well.
Model systems used in this thesis contributed to better understanding the role of structure activity relationships of Cer in skin barrier function. The pieces of knowledge from this work could be used in the

topical application of Cer (e.g., Cer NH) in atopic dermatitis treatment.