ABSTRACT

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Title of diploma thesis: The study of model lipid membranes containing omega-hydroxylated ceramides

Acylceramides (EO-Cer) belong to a class of ceramides (Cer) with an ultralong acyl chain whose ω-hydroxyl group is esterified with linoleic acid. The importance of EO-Cer lies in the formation of the long periodicity phase (LPP) and the corneocyte lipid envelope (CLE), which are indispensable components for the skin functioning as a barrier. Disorders in EO-Cer biosynthesis are associated with insufficient production of CLE and LPP leading to many skin diseases, including some types of ichthyosis. One of the enzymes that is deficient in such ichthyoses is PNPLA1. Insufficient function of this enzyme disables ω-esterification with linoleic acid, and thus the formation of EO-Cer. On the contrary, their precursors, i.e. ω-hydroxylated ceramides (O-Cer), are cumulated.

The aim of this thesis was to prepare model membranes containing O-Cer and to study the effects of O-Cer on the lipid organization and barrier properties of model membranes. Within this thesis, two types of membranes were prepared – the first type were membranes composed of a mixture of cholesterol, fatty acids and very long Cer with non-physiologically increased concentration of EO and O-Cer to better understand the effects of these ultralong lipids, and the second type were membranes containing EO and O-Cer in concentrations similar to physiological values. In addition, the effect of temperature during membrane preparation was studied.

In membranes with non-physiologically increased content of EO and O-Cer (prepared at 90 °C), the addition of O-Cer to such membranes led, with a few exceptions, to improved barrier properties (reduction in flux values and decreased water loss through the membrane). Apart from the short periodicity phase (SPP) and separated cholesterol, all membranes contained LPP. However, when all EO-Cer were replaced with O-Cer,

the barrier properties of the membranes were significantly disrupted, and the flux of model drugs increased almost three times. We assume that the increase in membrane permeability was caused by the absence of LPP, which has been replaced with another lamellar phase with a short periodicity. Decreasing the sample preparation temperature to 70 °C had a fundamental effect on the membrane lamellar arrangement. Membranes containing EO-Cer with addition of O-Cer or with increased concentration of EO-Cer contained only LPP (and separated cholesterol). Compared to the control membrane, no significant changes in permeability were found. Significantly difference results were again observed for the membrane, where all EO-Cer were replaced with O-Cer. Instead of LPP, the medium lamellar phase was found in this membrane. This lamellar arrangement caused a dramatic decrease in permeability compared to the membrane prepared at 90 °C, and the values were comparable with the control. We found that the membrane preparation temperature had a significant effect on both the lipid organization and the barrier properties of the membranes.

In membranes with physiological content of EO and O-Cer, we found the minimal EO-Cer concentration required for the LPP formation - 7.5 %. A complete replacement of EO-Cer with O-Cer or a decrease in the amount of EO-Cer below 7.5 % led to a disappearance of the LPP and only SPP was present. In membranes without LPP, a deterioration in barrier properties (increased fluxes and greater water loss) was observed (with a few exceptions). Compared to the previous membranes, the differences were not so pronounced. However, we again believe that the reason might be the absence of LPP.

In skin diseases with insufficient EO-Cer biosynthesis, where EO-Cer are replaced with their precursors, LPP is not formed. This may be one of the reasons why the skin barrier in these patients is deteriorated. However, there are other causes leading to an impaired skin barrier, such as insufficient synthesis of CLE, so it is not possible to consider changes in lipid composition separately as the essence of these diseases, it is necessary to study the whole issue comprehensively.