Abstract

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Title of doctoral thesis: Synthesis of human ω-*O*-acylceramides and evaluation of their effects on barrier properties of skin lipid membranes

The main objective of this work was to synthesize and study human acylceramides (acylCer, ceramides of the EO type) with the focus on the relationships between the acylCer concentration , structure of their polar head, their ability to form lamellar phases and permeability of model lipid membranes. Another objective was to synthesize ceramide-1-phosphates for the study of their signaling role in skin barrier and an analogue of ganglioside GM1 with shorter acyl chain as a standard for analysis of these lipids.

Ceramides (Cer) belong to the family of sphingolipids. They are the central molecules in sphingolipid metabolism, they participate on cell regulatory processes and the formation of human skin lipid barrier. The main skin barrier is situated in the *stratum corneum* (SC), which is the uppermost layer of skin. The purpose of SC is to maintain homeostasis of the inner environment and to prevent the penetration of exogenous substances, allergens and bacteria to the organism.

AcylCer are composed of sphingoid base, which is acylated by ultra-long (≥ 30C) acid, esterified by linoleic acid at its ω-hydroxyl group. AcylCer are the longest Cer occurring in the skin and they are essential for the proper function of the skin. Their complete deficit is lethal due to immense water loss through the skin. Healthy skin barrier contains approximately 9% of acylCer (of all Cer). Disturbances in the acylCer levels (mostly decreased levels) were found in several severe skin diseases, such as atopic dermatitis, psoriasis of lamellar ichthyosis.

Due to commercial unavailability of acylCer, we needed to develop and optimize their complete synthesis. The starting compound for this synthesis was 16-bromohexadecanoic acid. This acid served as a precursor for the preparation both the phosphonium salt and aldehyde, two reactants for Wittig reaction, which was a key step in this synthesis that enabled the preparation of the ultra-long chain. Resulting 32C unsaturated acid was converted to succinimidyl ester, which

increased its solubility and simultaneously served as a protecting group during its acylation with linoleic acid. Linoleic acid was attached to the ultra-long hydroxyl acid using Yamaguchi esterification reaction. In the last step, succinimidyl ester of the acid reacted with sphingoid base resulting in the desired acylCer. 12-step synthesis with overall 11% yield (in tens of milligrams), and 7% (in gram amounts) was developed.

Next, Cer-1-phosphates and modified gangliosides were synthesized. Cer-1-phosphates were synthesized using interphase phosphorylation with $P(OCH_3)_3$, followed by the hydrolysis of methyl groups to obtain two Cer-1-phosphates based on sphingosine and dihydrosphingosine in the overall yields 34% and 45%. These compounds will be used for the study of their role in cell signalization during the formation of skin barrier and their behavior in lipid membranes. Ganglioside GM1 with shorter acyl chain was prepared as a standard for ganglioside quantification. Natural ganglioside GM1 was used as a starting compound. It was enzymatically deacylated and then acylated again with an acid with shorter chain to obtain the product in 40% overall yield.

The prepared acylCer were used for the preparation of model lipid membranes mimicking the skin lipid barrier. Two series of the membranes were prepared – a simple and complex model. The simple model was composed of one acylCer (Cer EOS)/one Cer (Cer NS)/ lignoceric acid/cholesterol with addition of cholesteryl sulfate. Using X-ray powder diffraction we studied the concentration of Cer EOS necessary for the formation of the long periodicity phase (essential for the proper barrier function of the skin). It was found, that this phase appears at 10% concentration of Cer EOS and coexists with the short periodicity phase. At 30% Cer EOS, the short periodicity phase disappeared. Though in permeability experiments, this model showed to be too simple and the addition of acylCer to the membrane led to increase in the permeability to model compounds.

To obtain results relevant to *in vivo* findings, the simple model was substituted by more complex model that contained different acylCer (or their mixture), mixture of Cer, mixture of fatty acids, cholesterol and an addition of cholesteryl sulfate. Experiments with complex model were focused on the comparison of different structures of acylCer polar head. The permeability of all membranes of the complex model containing acylCer was decreased compared to control (without acylCer), which correlates much better with the experiments using human skin. However, X-ray powder diffraction measurements showed that only the most complex membrane with the mixture of acylCer forms the long periodicity phase. These results for the first time describe the relationships between the acylCer structure and their behavior in lipid membranes. In the future, this model will be used in preparation of the models of lipid barrier in skin diseases and to study compounds with potential ability to regenerate impaired skin barrier.

The prepared acylCer were also used as standards for the quantification of acylCer in biological material. Using HPTLC, we were able to prove that the prepared acylCer occur in skin. We also improved their quantitative analysis and found that identical standards are necessary for their proper quantification. Using incorrect standards may lead to errors reaching up to hundreds percent. AcylCer were also used for quantification of lipids in reconstructed skin with filaggrin knockdown as a model of atopic dermatitis. It was found that peroxisome proliferator activated receptor α agonists improve skin homeostasis, increase skin barrier function and normalize the composition of free fatty acids.

To conclude, an efficient, scalable and reliable synthesis of human ultra-long Cer was developed. The synthesized acylCer were used for the preparation of model lipid membranes simulating healthy or impaired skin, which opens new possibilities of *in vitro* evaluation of potential drugs for the treatment of severe skin diseases, such as atopic dermatitis or psoriasis. These acylCer were also used to improve quantitative analysis of lipids in skin samples.