Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of inorganic and organic chemistry

Candidate**: Mgr. Barbora Školová**

Supervisor: **doc. PharmDr. Kateřina Vávrová, Ph.D.**

Title of doctoral thesis: **Synthesis and study of ceramide analogues**

Ceramides (Cer) together with free fatty acids and cholesterol form intercellular lamellar space of the uppermost skin layer (*stratum corneum*, SC). **This lipid matrix presents the proper skin barrier** – it protects organism against outer environment and keeps its homeostasis as well. The composition and arrangement of the intercellular lipid matrix is essential for the skin barrier properties. In spite of this fact, **the organization of SC lipids at the molecular level is not fully elucidated**. The aim of this work is to contribute to more detailed insight into organization of skin barrier using SC lipid model membranes, in particular to find **the structural parameters in ceramide molecules**, which play a role in the maintenance of the skin barrier. The SC model membranes present a useful tool to study SC lipids, they mimic physiological or pathological conditions in the skin. The obtained results should be used in diagnosing or therapy of skin diseases characteristic with impaired barrier function, such as atopic dermatitis or psoriasis.

Firstly we focused on explanation of the mechanism of loss of the skin barrier function due to the **shortening of Cer acyl**. We studied permeability of SC lipid membranes and their biophysical properties by differential scanning calorimetry (DSC), infrared (IR) spectroscopy and powder X-ray diffraction and also we evaluated the lipid monolayers (by Langmuir isotherms and atomic force microscopy). At the skin temperature (i.e. 32°C), **in all membranes** (including the most permeable membranes based on Cer with 4 and 6C acyls) **lipid chains are well ordered** (mainly in all-*trans* conformation and in orthorhombic lateral packing). In membranes containing short-acyl Cer the **separation** of lipid domains and the presence of **short-lamellar phases** were observed, which could be reasons of their higher permeability. Monolayers of the mixture containing native Cer (with 24C acyl) are arranged spontaneously and on solid support they form larger continuous domains in larger area.

In skin of atopic dermatitis patients increased levels of long Cer (C16) at the expense of the native Cer with very long acyl (C24) were found. Thus, we decided to study the behavior of SC lipid membranes containing mentioned Cer by DSC, IR and solid-phase ^{2}H NMR (nuclear magnetic resonance) spectroscopy. Before the preparation and evaluation of lipid membranes Cer with deuterated acyls were synthesized. Unlabeled and deuterated compounds could be in mixtures studied simultaneously but separately due to different positions of absorption bands in IR spectra. We found that in model SC lipid membranes the native Cer (C24 acyl) adopts the **extended conformation** with chains pointing in the opposite direction. This unusual conformation would increase cohesion of the lipid lamellae and could explain the low permeability of native Cer membranes. At the skin temperature (i.e. 32°C), the very long Cer (C24) is **crystalline** in model membrane but the dominating phase of long Cer (C16) is more permeable **gel state**.

Previous studies suggested that a specific **ratio of dihydrosphingosine Cer** (dihydroCer) **and sphingosine Cer** (sphCer) is important for the skin barrier properties. We decided to study the importance of *trans*-

double bond in SC membranes by permeability experiments, DSC, IR and ^{2}H NMR spectroscopy and we also aimed at studying the effect of acyl chain length (C 2, 4, 6, 8, 24). We found higher (but not significantly) membrane permeability of short-acyl dihydroCer compare to native (24C acyl) dihydroCer. Compared to dihydroCer with shortened acyl chains significantly higher permeabilities were found in the short-acyl sphCer membranes. However, membranes containing native dihydroCer are more permeable than membranes based on native sphCer. It could be explained by **different proportion of very tight orthorhombic lateral packing**, which is lower in sphCer membranes. At the skin temperature (32°C), increased portion of **isotropic phase** of the native dihydroCer (C24 acyl) in lipid membranes was detected (27%) compare to 1% of this phase of sphCer (C24 acyl).

The **length of sphingosine chain** also influences skin barrier properties. Cer analogues with shortened sphingosine (to 12 and 15C) and acyl (to 2, 4 and 6C) chains increase permeability of skin and SC model membranes as well. The biophysical properties of these membranes studied by IR spectroscopy differ slightly, e.g. lipid chains are well ordered. The differences in **amide vibration** indicate different hydrogen-bonding interaction although Cer have the same arrangement in the polar head region. Together with it lots of **short lamellar phases** in the most permeable membranes were identified, which could be reason for their higher permeability.

The obtained results improved our knowledge of SC lipid properties and contributed to better understanding of skin barrier function. The influence of several structural features in Cer molecules on the barrier properties and behavior of SC lipid membranes was elucidated, which could be used e.g. in design of skin barrier repair agents.