ABSTRACT

The extracellular matrix of the uppermost layer of the skin, the *stratum corneum* (SC), consists of ceramides (Cer), cholesterol (Chol), free fatty acids (FFA) and cholesteryl sulfate (CholS). Cer play an important role in the correct barrier function of mammalian epidermis. A new type of sphingolipids, *i.e.*, 1-*O*-acyl-Cer, have been found in human SC very recently; however, their role in the SC is unknown. These Cer species contain sphingosine (S) that is *N*-acylated with non-hydroxylated or α- hydroxylated fatty acid, and moreover, hydroxyl group at C1 in sphingosine is esterified by an additional fatty acid (lignoceric acid, C24 or palmitic acid, C16). Because 1-*O*-acyl-Cer are not commercially available, we aimed to synthesize physiological 1-*O*-acyl Cer, *i.e.*, Cer-24NS16, Cer-16NS16 and Cer-24AS16. Moreover, we aimed to study their behaviour on permeability and microstructure of model skin lipid membranes.

The 1-*O*-acyl-Cer were synthesized by an acylation of Cer-NS16 or Cer-AS16 with palmitic or lignoceric acid using *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide and 4- dimethylaminopyridine. Cer-AS16 was prepared by α-bromination of palmitic acid, substitution of bromine by hydroxyl and *N*-acylation of sphingosine by the prepared acid in the presence *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide and 1-hydroxybenzotriazole.

Afterward, simple model SC lipid membranes were prepared with the following composition: Cer-NS24/FFA C_{16-24}/Chol/CholS as a control membrane and then 5, 10, 20, 30, or 100% of Cer-NS24 was replaced by Cer-24NS16. Next, the complex model SC lipid membranes were composed of isolated Cer from human SC/FFA C_{16-24}/Chol/CholS, in where 10% of SC Cer were replaced by Cer-24NS16, Cer-16NS16, Cer-24AS16 or Cer-24AS16-24. The permeability of model lipid membranes SC was assessed in Franz-type diffusion cells using the following permeability markers: flux of theophylline and flux of indomethacin, electrical impedance and water loss through the membrane. To elucidate the mechanisms of 1-*O*-acyl-Cer on skin permeability, their effects on the membrane biophysics was investigated by infrared spectroscopy and X-ray powder diffraction.

From the results of permeation studies we found out that a 10% addition of 1-*O*-acyl-Cer in model membranes (simple model) led to decrease of permeability for water and ions. On the other hand, the presence of 10% of 1-*O*-acyl-Cer in complex model did not change the

permeability or slightly increased it. Using IR spectroscopy we found that the effect of 1-*O*-acyl-Cer on the conformation of the lipid chains, phase transitions and lateral arrangement of lipids in model membranes is not very significant. Lamellar arrangement of model lipid membranes was further evaluated by X-ray powder diffraction. In all studied membranes was present phase of separated Chol and short lamellar phase with a periodicity of approximately 5.3 to 5.7 nm.

This work provides the first characterization of 1-*O*-acyl-Cer, as the components of extracellular matrix SC. These lipid do not affect or slightly deteriorate the barrier function of studied membranes with the human Cer; between different structural types of 1-*O*-acyl-Cer we did not observe significant differences.