Abstract

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Title of diploma thesis: **Study of cholesterol depletion in human skin barrier**

Barrier function of human skin barrier dwells in intercellular lipid membranes of the uppermost skin layer, the *stratum corneum* (SC), composed of equimolar mixture of ceramides (Cer), free fatty acids (FFA) and cholesterol (Chol).[1] Chol is required for proper lipid organization of SC, however, it stays unclear, why is it present in an amount so high that it separates from other lipids.[2] Experiments using synthetic model membranes with decreased Chol content suggested that molar ratio of Cer:FFA:Chol 1:1:0,4 is sufficient for lipid barrier formation and its complex functionality.[3]

The aim of this work was to manipulate Chol content directly in human SC and to study the effects of decreased Chol content on the SC permeability and microstructure.

Ex vivo SC obtained from healthy donors was extracted by methyl-β-cyclodextrin (MβCD) to reduce natural Chol content. The extracted SC did not show significant changes in Cer or FFA whilst the amount of Chol was lowered to 78 %. SC barrier properties were evaluated by measurements of transepidermal water loss (TEWL), electrical impedance (EI) and permeabilities for theophylline (TH) and indomethacin (IND). Significant difference between TEWL of CD-extracted and control sample was not detected. Decreased EI and permeability to TH, and a slight increase in permeability to IND were found. That corresponds to synthetic membranes with similar Chol content, suggesting that barrier function of SC with lower than natural amount of Chol is not significantly impaired. Molecular organization, investigated using infrared spectroscopy, did not reveal significant changes. Furthermore, small angle X-ray diffraction suggested that the MβCD treatment decreased the intensity of the separated Chol while that of a SC mixed lipid phase appeared relatively stable. Our results suggest that the separated Chol domains are not necessary for the SC barrier formation and function.

The study was supported by the Czech Science Foundation (16-25687J) and Charles University (GAUK 936216 and SVV 260 401).