Abstract

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Title of doctoral thesis: Study of barrier lipids in the skin and skin models

The barrier lipids are found in the intercellular spaces of the uppermost layer of epidermis – stratum corneum (SC). These lipids comprise an approximately equimolar mixture of ceramides (Cer), fatty acids (FA) and cholesterol (Chol). The composition and organization of the lipid mixture is unique and provides a barrier, which protects organism against harmful effects from the surroundings and, at the same time, it protects body from excessive water loss and contributes to homeostasis maintenance.

Skin barrier disruption, including altered barrier lipid composition and organization, was described in many skin diseases, for example atopic dermatitis, psoriasis or skin cancer. Study and therapy of these diseases are limited by their complex pathophysiology and a lack of biological material (human skin). Therefore, skin models are appropriate and useful tools for study of skin diseases. The skin models differ in their simplicity/complexity and their preparation therefore they can provide different information.

Model lipid membranes are simple models of intercellular lipid matrix of SC. First prepared model membranes simulated a disrupted Cer metabolism in SC with Gaucher disease. In healthy state, Cer arise from their precursors – glucosylCer (GlcCer), in the diseased state, however, the Cer transformation is reduced or disabled. Model membranes with Cer fraction partially replaced by GlcCer were prepared. Membranes permeability was studied using four permeability markers and membrane microstructure was assessed by X-ray powder diffraction. Replacement of small amount of Cer by GlcCer led to impaired barrier properties and altered lipid organization of the membrane. By contrast, replacement of 50% and more caused formation of a novel lamellar organization and the membranes had better barrier properties in comparison to control membrane. The results suggest that accumulation of GlcCer is not the main cause of increased transepidermal water loss in patients with Gaucher disease.

Also using model lipid membranes, the influence of Chol amount in the lipid matrix on barrier properties was studied. X-ray powder diffraction showed that approximately only half of the equimolar amount of Chol is mixed with other lipids and integrates in the lamellar organization. More than half-amount of Chol creates additional separated phase in the membrane structure. The membrane permeability was not negatively influenced by Chol reduction, by contrast, the membrane with 40% Chol had the best barrier properties. This implies that the equimolar amount of Chol has probably other than barrier function in the SC.

Reconstructed skin equivalents (RHS) are more complex model systems. They contain dermal and epidermal cells, which create a stratified epidermis. These models are suitable for biochemical analyses, where usually influence of a gene mutation or addition of specific

cells is investigated. Mutations in the gene encoding for filaggrin (FLG) are the major predisposing factor for atopic dermatitis. RHS with normal and decreased FLG expression were prepared at cooperating institution in Germany. FLG-deficient models have higher content of FA in SC and worse lipid organization compared to the control model. Influence of selected PPAR agonists on lipid composition and organization of SC in prepared RHS was evaluated. Selective PPARα agonist increased expression of FLG and other structural proteins, normalized level and composition of FA, which led to improved lipid organization and barrier properties of FLG-deficient RHS. Therefore, PPAR agonists could be used in treatment of FLG-associated skin diseases.

Since atopic dermatitis is a chronic inflammatory disease, FLG-deficient RHS with addition of activated immune cells were studied. Impact of immune cells on homeostasis and skin barrier of the models was observed. Increased level of pro-inflammatory cytokine TSLP was found in the FLG-deficient model, TSLP was increased after addition of immune cells also in the control models. Both types of models showed higher permeability and increased values of surface pH. Exclusively in FLG-deficient models, the migration of activated immune cells from the application site into the dermal compartment was noted. This finding indicates that TSLP directly triggers immune cells migration, which had not been reported before.

RHS could be a valuable tool for searching and designing targeted skin cancer treatment. For that purpose, reconstructed models of skin cancer (in different stages) were prepared in Germany and their barrier properties were characterized at our Department. The analysis revealed impaired lipid organization as well as altered composition of the lipid matrix. Skin barrier defect and increased permeability of models correlated with stage of skin cancer (number of carcinoma cells). The prepared RHS mimic well the local changes of skin barrier of skin cancer lesions in vivo.

Human skin *ex vivo* (full-thickness or its individual layers) can be used in many experiments. Full-thickness skin was used in permeation experiments to compare the results obtained from experiments with model lipid membranes. Topical application of GlcCer on intact *ex vivo* human skin disrupted the skin barrier, represented by higher values of transepidermal water loss and lower values of electrical impedance. In the second study, selective extraction of Chol from isolated human SC confirmed results of model membranes with the same Chol proportion. Although only 20% of Chol were extracted from human SC, comparable values from both model lipid membranes and extracted SC indicate a suitability and reproducibility of model membranes in this kind of study.

Skin sampling from patients with skin diseases is a limiting step in diagnostics and research of skin diseases. Non-invasive techniques of skin sampling could strongly improve patient's comfort in comparison to punch biopsy. Therefore, two skin sampling techniques were compared (in cooperation with Dr. Svoboda) – tape stripping and suction blistering (SB). SB provides an intact epidermis in sufficient amount for isolation of proteins and RNA, for preparation of histological sections and also for qualitative and quantitative analysis of barrier lipids.

The results of the work show practical application of the skin models of different complexity in scientific studies. Acquired knowledge contributed to better understanding of the function of barrier lipids in healthy and diseased skin.