SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of certain aspects of the cutaneous barrier

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The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 1 pm, 29th September 2025.

Introduction and literature review

Our research group has also investigated the role of transient receptor potential (TRP) ion channels in the skin. Among other things, we have shown that the heat-sensitive TRPV1, -2, and -4 ion channels are expressed in human sebocytes. The first part of this thesis rooted from a recent study in which our collaborators described that TRPM5, a member of the TRP superfamily, could be a promising therapeutic target for hair growth disorders. This publication raised the possibility that targeting this channel may influence biological processes of sebocytes, and hence, may lead to the development of potential sebaceous gland-related side effects. Thus, we aimed to investigate the expression and functional role of TRPM5 in human sebocytes.

In the second part of the thesis, we continued a previous project and investigated the effects of a selective serotonin reuptake inhibitor, fluoxetine (FX), on human epidermal keratinocytes. Our research group has previously shown that FX, an antidepressant that has been used in the clinical practice for many years, reduced the polyinosinic polycytidylic acid [p(I:C)]-induced inflammatory response and endothelin release in human epidermal keratinocytes via indirect inhibition of the phosphoinositide 3-kinase (PI3K) pathway, when applied at 14 μM. Since the PI3K pathway is not only involved in mediating inflammatory responses, but it is also a positive regulator of proliferation, our experiments aimed to investigate the effects of the potent anti-inflammatory concentration of FX (14 μM) on the proliferation, differentiation, and barrier-forming capacity of human epidermal keratinocytes.

The epidermis: keratinocytes and barrier function

Keratinocytes are key players in the formation of the epidermal barrier. First, they proliferate in the innermost layer of the epidermis, then they move towards the surface. They undergo characteristic morphological changes during their differentiation process, creating the typical stratified histological pattern of the epidermis. During this tightly regulated process (which ultimately leads to the loss of the nuclei and the apoptosis of the cells), keratinocytes express various proteins that are specific to the particular phase of the process, and hence, can be used as differentiation markers in research. The cells of *stratum basale* are characterized by the expression of keratin (K) 5 and 14. Then, from the beginning of differentiation,

when the cells move to the *stratum spinosum*, they start to express involucrin in addition to K1 and K10. The *stratum granulosum* is characterized by the presence of loricrin and profilaggrin (a precursor of filaggrin) packaged in keratohyalin granules. The proteins and other intermediate filaments formed during this process maintain the stability of the upper layers of the epidermis and are involved in the formation of several cell-cell contacts, including tight junctions. In addition to claudins, occludin (OCLN) is important in tight junction formation and is also used as a marker of keratinocyte differentiation status.

The intercellular space contains epidermal lipids as well, which are formed during keratinocyte differentiation. The continuous production of cholesterol, ceramides, free fatty acids, and other lipids, together with the surface sebum, not only stabilize the corneocytes, but also provide additional insulation and physical protection to the skin.

Biology of sebaceous glands and sebocytes, the lipid barrier

Sebaceous glands and their cells, the sebocytes, which are responsible for the production of sebum, are integral parts of the complex cutaneous barrier and the immune system.

The sebum is produced by holocrine secretion, and it contains mainly neutral lipids. Due to its unique composition, sebum plays a role in maintaining the skin barrier on several fronts. The immunocompetent nature of sebocytes and their role in immunological defense is supported by the expression of several pathogen-associated molecular pattern recognizing receptors (including e.g., CD14, TLR2, -4, and -6) and their involvement in inflammatory processes. They are able to produce various antimicrobial peptides and lipids, as well as inflammatory cytokines. Indeed, expression of interleukin (IL)-1α, IL-1β, IL-6, IL-8 (CXCL8), and tumor necrosis factor (TNF)-α has been described both *in vivo* and *in vitro*.

The transient receptor potential (TRP) ion channel superfamily and TRPM5

TRPs are a heterogeneous family of ion channels with diverse functions and are expressed in a wide range of cell types. Based on their structural and sequence homology, vertebrate TRP channels can be divided into 7 subfamilies: classical or canonical (TRPC), vanilloid (TRPV), ankyrin (TRPA), polycystin (TRPP),

mucolipin (TRPML), “no mechanoreceptor potential C” (NOMPC or TRPN), and melastatin (TRPM).

The melastatin subfamily is composed of multifunctional cation channels with diverse physiological properties, and its individual members can be expressed in multiple isoforms. Alternative mRNA splicing significantly influences the biophysical properties of the channels and can even result in functionally inactive variants. One important member of the family is TRPM5, which is a Ca^{2+}-activated ion channel that is only permeable to monovalent cations (Na^{+} and K^{+}). Like many other TRP channels, TRPM5 is a thermosensitive, heat-activated channel.

TRPM5 is involved in taste sensation, it is expressed in the respiratory system, as well as on chemosensory cells of the olfactory system, and it is a regulator of insulin secretion. In addition, the activity of the channel also affects the human hair cycle. Commonly used modulators (that have also been used in our experiments) of TPRM5 include the antagonist triphenylphosphine oxide (TPPO), and the activators 2-heptanone (Hept) and 2,5-dimethylpyrazine (DMP).

TRP channels in the human skin

TRP channels are expressed in many cell types of the skin and skin appendages, including keratinocytes, sebocytes, melanocytes, sensory neurons, and hair follicles. Their role in the skin is complex and diverse; the channels can affect, for example, keratinocyte proliferation and differentiation, barrier formation, sebaceous lipid production, inflammatory processes, and hair growth.

Our research group has previously investigated the relationship between sebocytes and TRP channels. We have previously shown that TRPV1, TRPV2, TRPV3, and TRPV4 were expressed in human sebocytes, whereas the mRNA expression of TRPA1 and TRPM8 were below the detection limit in SZ95 human sebocytes.

Recently, our collaborators have investigated the role of TRPM5 in human hair follicles. In their experiments, they found that TRPM5 was expressed in human hair follicles and its homeostatic activity played a role in maintaining the growing (anagen) phase of the hair cycle, making TRPM5 a promising therapeutic target in the treatment of diseases associated with unwanted hair growth or involuntary hair loss. Because the immunofluorescent images published in said article indicated that

sebaceous glands also exhibited TRPM5 positivity, one might speculate that TRPM5 modulators administered with the intention of influencing hair growth may also have an unintended and unexpected impact on sebaceous gland functions. Therefore, in one of our publications that formed the basis of this dissertation, we investigated the expression of TRPM5 and the effects of available TRPM5 modulators on human sebocytes.

Dermatological repositioning of the antidepressant fluoxetine (FX)

Fluoxetine (FX) is a widely used selective serotonin reuptake inhibitor (SSRI) that is usually administered as an antidepressant. Interestingly, over the years, several its several additional beneficial effects have also been revealed. Indeed, it can increase melanin production, promote repigmentation of microdissected human hair follicles, and even it has a positive effect on wound healing when applied topically, which has been confirmed in diabetic mice and in infected wounds in humans. In addition, it has been proven in many cases that FX can also induce anti-inflammatory and antipruritic effects.

Considering the above data, our research group also became interested in the topic of the possible dermatological use of FX. In our recently published experiments, we examined the effects of the highest non-cytotoxic concentration of FX (14 μM) on cultured human epidermal keratinocytes. We found that at this concentration, FX significantly reduced the inflammatory response induced by the TLR3 activator p(I:C) and the release of the endogenous itch mediator endothelins by indirectly inhibiting the PI3K pathway. It is known that PI3K signaling, in addition to regulating inflammatory processes, also enhances the proliferation of epidermal keratinocytes, which raised the possibility that FX may exert an anti-proliferative effect on human epidermal keratinocytes over the course of long-term application, and, by changing the proliferation/differentiation balance, it may affect keratinocyte differentiation and hence the formation of the epidermal barrier.

Aims

Based on the above literature data and on our previous results, we aimed to answer the following questions:

1. Is TRPM5 expressed in a functionally active form in human sebocytes?
2. What are the biological effects of TPRM5 modulators on human sebocytes?
3. What are the effects of a promising, anti-inflammatory concentration of FX (14 μM) on the proliferation and differentiation of human epidermal keratinocytes?

Materials and Methods

SZ95 human immortalized sebocytes

SZ95 sebocytes (*ID of the Material Transfer Agreement that enabled the use of SZ95 sebocytes: SZ95-A 236-1*) were cultured in Sebomed Basal Medium® supplemented with the following: heat-inactivated fetal bovine serum (FBS), $CaCl_2$, human recombinant epidermal growth factor, amphotericin B, MycoZap™ Plus-CL. Cells were cultured in a humidified 5% CO_2 incubator at 37 °C, with medium changes every two days. *Cell culture was performed by József Arany, Tamara Nyitrai, Mónika Barotáné Kovács, Orsolya Pető, Beatrix Tolvaj, and Dorottya Ádám-Nagy.*

HaCaT human immortalized epidermal keratinocyte cell line

HaCaT keratinocytes were cultured in "low Ca^{2+}" Dulbecco's Modified Eagle Medium (DMEM). The medium was supplemented with heat-inactivated FBS, MycoZap™ Plus-CL, and L-glutamine. The cells were cultured in a humidified 37 °C incubator with 5% CO_2, medium was changed every two days, and then the cells were passaged when they reached 70–80% confluence. In some of our experiments, when the cells reached 100% confluence, another DMEM with a higher Ca^{2+} concentration (supplemented with heat-inactivated FBS and MycoZap™ Plus-CL) was used to induce differentiation. *Cell culture was performed by dr. Fanni Kinga Tóth Volascsekné, Mónika Kovács Barotáné and Dorottya Ádám-Nagy.*

Primary normal human epidermal keratinocytes (NHEK)

Primary normal human epidermal keratinocytes (NHEKs) were isolated from dermatologically healthy individuals who had undergone surgical intervention. Before skin removal, volunteer donors were properly informed and gave written informed consent for the use of the samples for research purposes. The experiments were approved by the Regional and Institutional Research Ethics Committee of the University of Debrecen and the Hajdú-Bihar County Government Office (IDs: *61566-5/2021/EÜIG, DE RKEB/IKEB 4988-2018*), in compliance with the guidelines of the Declaration of Helsinki.

Primary keratinocytes isolated from skin samples were cultured in appropriately supplemented EpiLife medium. The cells were cultured in a humidified 37 °C incubator with 5% CO_2. Medium was changed every two days, and then the cells were passaged when 60-70% confluence was reached. *The isolation and culture of the cells was performed by Dorottya Ádám-Nagy.*

Experiments performed in a reconstructed epidermis-equivalent model

After 3D culturing, 6 μm paraffin sections were prepared from the samples for further studies (hematoxylin-eosin [HE] staining, keratin [K]-1 and K10 as well as Ki-67 immunolabeling). Semi-quantitative image analysis was performed using *ImageJ 1.51j8* software using images taken at 300× original magnification. *The cultures were established and processed by Dorottya Ádám-Nagy under the supervision of dr. Hanna Niehues and prof. dr. Ellen van den Bogaard. HE staining, K1 and K10 labeling as well as LY assay were performed by Dorottya Ádám-Nagy. Evaluation was performed by dr. Attila Oláh and Dorottya Ádám-Nagy. In case of LY dye penetration assay, the images were taken by dr. László Szabó, whereas Ki-67 labeling was performed by dr. Ágnes Pór. Evaluation was performed by dr. Attila Oláh and Dorottya Ádám-Nagy.*

RNA isolation, reverse transcription, and quantitative, real-time polymerase chain reaction (RT-qPCR)

For reverse transcription, the RNA samples were diluted to a predetermined, equal concentration, and then cDNA was prepared from our samples using the High

Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, according to the manufacturer's protocol.

The mRNA expression was determined by real-time polymerase chain reaction (RT-qPCR) using 5' TaqMan™ Gene Expression Assays and TaqMan™ Gene Expression Master Mix following the manufacturer's protocol, and a Roche LightCycler 480 System was used for detection. The relative expression of each gene was normalized to the gene expression values of these housekeeping genes using the ΔCT method or, in some cases, normalized to the expression of the housekeeping gene and then to the relative gene expression in the control group using the ΔΔCT method, and given as mean±SD. *The experiments related to the RT-qPCR were performed by Dorottya Ádám-Nagy.*

Selective gene silencing with small interfering RNA (siRNA transfection)

Selective gene silencing of SZ95 sebocytes was performed in serum-free Opti-MEM® medium using Lipofectamine® RNA_i MAX transfection reagent according to the manufacturer's protocol. The efficiency of silencing was assessed at the protein level by western blot. *The selective gene silencing was performed by Dorottya Ádám-Nagy.*

Western blotting

Equal amounts (20 μg) of samples were loaded per lane onto a gel. Following the electrophoresis, the proteins in the gels were transferred to a nitrocellulose membranes.

After blocking, the membranes were incubated overnight at 4 °C with TRPM5- or EGFR-specific primary antibodies. On the next day, the membranes were washed and then incubated for 1 hour at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies compatible with the primary antibody. Immunoreactive bands were visualized using chemiluminescent SuperSignal™ West Pico PLUS or SuperSignal™ West Femto Maximum Sensitivity Substrate kits on a KODAK Gel Logic 1500 Imaging System using *Kodak MI 4.0.5* software. To assess equal loading in case of siRNA transfections, β-actin was used as "loading" control. The specificity of one of the TRPM5-specific antibodies was also checked with the blocking peptide recommended by the manufacturer. When necessary,

semiquantitative densitometric analysis of the western blot bands was performed using *ImageJ 1.51j8* software. *Western blotting was performed by József Arany and Dorottya Ádám-Nagy.*

RNA-Seq method

To collect global transcriptome data, only samples with an RNA integrity number (RIN) >7 were used for mRNA library preparation. The raw data of the RNA-Seq analysis are available in the NCBI SRA database (identifiers: PRJNA1122410, and PRJNA1037731, respectively). Principal component analysis and pathway analysis were performed using the *StrandNGS software*, the *CytoScape v3.4* and *ClueGo v2.3.5.* applications.*The RNA samples were prepared by dr. Fanni Kinga Volascsekné Tóth and Dorottya Ádám-Nagy. Sequencing and data evaluation were performed by dr. Szilárd Póliska.*

Cytokine release (ELISA)

To determine IL-6 and IL-8 (CXCL8) release from the cells, supernatants were collected following 3- and 24-h treatments, and detection was performed using BD OptEIA Kits. *The experiments were performed by Dorottya Ádám-Nagy.*

Phosphokinase array

For the phosphokinase array, the Proteome Profiler Human Phospho-Kinase Array Kit was used according to the manufacturer's protocol. The membranes were incubated with identical protein amounts. The average signal intensities of the negative reference spots on each membrane were considered as background intensity, and were subtracted from the raw values. The obtained results were then normalized to the values of the control cultures, and a ≥1.3-fold increase was considered to be a relevant alteration. *The phosphokinase array was performed by József Arany and Dorottya Ádám-Nagy.*

Investigation of cellular viability (MTT-assay)

After 24 or 48 hours of treatments, the supernatant was removed from the cells, and MTT solution (0.5 mg/ml) was added to them. The plates were then placed were incubated at 37 °C for 2.5 hours. The supernatant was then removed and the crystals were dissolved by adding MTT solubilizing solution and shaking at room temperature. The absorbance of the resulting homogeneous solution was measured

at 565 nm using FlexStation 3 plate reader. *The MTT-assay was performed by Tamara Nyitrai, Orsolya Pető, and Dorottya Ádám-Nagy.*

Quantification of the intracellular lipid content (Nile Red labeling)

After 24 or 48 hours of treatments, the supernatant was removed from the cells and 1 μg/ml Nile Red working solution was added to each well. After 30 minutes of incubation (at 37 °C), the fluorescence intensity was measured using a FlexStation 3 device. Neutral lipids were detected at 485 nm excitation and 565 nm emission wavelengths, while polar lipids were assessed using 540 nm excitation and 620 nm emission wavelengths. *The Nile Red labeling was performed by Tamara Nyitrai, Orsolya Pető, and Dorottya Ádám-Nagy.*

Investigation of proliferation (CyQUANT-assay)

Keratinocytes were plated on black well clear bottom 96-well plates for the CyQUANT proliferation assay at a density of 20,000 cells/well. After executing the necessary treatments, the supernatant was removed from the wells and the plates were placed at -80 °C for at least overnight to facilitate cell permeabilization. The assay was performed following the manufacturer's protocol and the intensity of the fluorescent signal was detected using a FlexStation 3 device (excitation/emission wavelengths: 480/520 nm). *The CyQUANT-assay was performed by Dorottya Ádám-Nagy.*

Examination of the intracellular ion homeostasis

Fluorescent Ca^{2+}-measurement (Fura-2 AM labeling)

The cells were incubated with the 2 μM Fura-2 AM at 37 °C for 30 minutes. The fluorescent signal was monitored using excitation wavelengths of 340 nm and 380 nm, and an emission wavelength of 510 nm. To investigate whether TRPM5 modulation indirectly affected Ca^{2+} influx through TRPV4 (a Ca^{2+}-permeable TRP channel also expressed on sebocytes), a TRPV4 agonist (GSK1016790A) was administered as the last step of the measurement. *The fluorescent Ca^{2+}-measurement was performed by Dorottya Ádám-Nagy.*

Fluorescent Na^{+}-measurement (SBFI AM labeling)

Cells were loaded by using 10 μM SBFI AM, a Na^{+}-sensitive fluorescent dye. Cultures were incubated with the dye for 40 min at 37 °C. The fluorescent signal was

detected at 37 °C (excitation wavelengths: 340 nm and 380 nm; emission wavelength: 500 nm). *The fluorescent Na^+-measurement was performed by Dorottya Ádám-Nagy.*

Real-time measurement of electrical impedance of HaCaT keratinocytes

The real-time monitoring of the electrical impedance of the HaCaT cells was performed using the xCELLigence system. When full confluence was reached, the medium was replaced with differentiation-inducing, "high-Ca^{2+}" DMEM (see above) that contained 14 μM FX or an equal volume of vehicle (DMSO). *The cells were prepared by Dorottya Ádám-Nagy, and the measurement was performed by dr. Judit Váradi. The data evaluated by dr. Attila Oláh and Dorottya Ádám-Nagy.*

Statistical Analysis

Data were analyzed by GraphPad Prism *10.1.0 (316)*. Outliers were identified by using the ROUT method (Q = 1%). Depending on the sample size, Gaussian distribution was tested by Anderson-Darling or Shapiro–Wilk tests. In case of Gaussian distribution, Student's two-tailed, unpaired *t*-test (paired comparisons), one-way ANOVA followed by Šidak's or Dunnett's multiple comparisons test were used, whereas in case of non-Gaussian distribution, two-tailed Mann–Whitney test (paired comparisons) or Kruskal–Wallis test followed by Dunn's multiple comparisons test were used. Fisher's exact test was used to analyze Ki-67 positivity in individual donors. $P < 0.05$ values were regarded as significant differences. *The statistical analysis and the preparation of the figures were performed by dr. Attila Oláh and Dorottya Ádám-Nagy.*

Results

Expression of TRPM5 is dubious in human sebocytes

At the beginning of our experiments, we wanted to confirm the presence of TRPM5 on human sebocytes using preconfluent (i.e., actively proliferating) and postconfluent (i.e., spontaneously differentiated) cells. Although we employed two different TRPM5-specific TaqMan assays, we found that mRNA expression of the ion channel remained around or below the detection limit in all cases.

Next, further expression analysis was carried out at the protein level using western blotting. In the first experiments, a specific blocking peptide was used in addition to the antibody. This experiment resulted in several apparently non-specific bands, most of which disappeared when the antibody was co-incubated with blocking peptide. Importantly, however, none of the bands appeared at the molecular weights predicted by the manufacturer (≈100 and 72 kDa), but weak band was seen slightly above the molecular weight predicted by the UniProt database (≈132 kDa). However, this band did not disappear in the presence of the blocking peptide.

Because of the appearance of several, most likely non-specific bands, we repeated the experiment by using another anti-TRPM5 antibody. In this case, the western blot resulted in a more "specific-looking" band; however, the immunopositivity did not appear at the molecular weight predicted by the manufacturer (≈100 kDa) but was located slightly above it (≈110 kDa). In the absence of appropriate blocking peptide, the specificity of this band was challenged by siRNA transfection. To this end, three TRPM5-specific siRNA constructs were used, but none of the constructs was efficient in significantly decreasing relative optical density of the western blot bands as compared to the non-sense RNA-transfected scrambled control (SCR) group.

Because the specificity of the western blot labeling was questionable, as a last step of our expression experiments, we also assessed the functional activity of TRPM5 in sebocytes. Since the TRPM5 ion channel is permeable to monovalent cations, in the functional study, we first tested how TRPM5 modulators affected the Na^+-homeostasis of the cells using SBFI AM, a Na^+-sensitive fluorescent dye.

To this end, we first checked how the known TRPM5 modulators affect the viability of sebocytes using the MTT-assay. We found that, up to a concentration of

1000 μM, neither the activators (DMP and Hept) nor the antagonist (TPPO) had a negative effect on the viability of SZ95 sebocytes. Based on the MTT-assay, we selected a high, but certainly non-cytotoxic concentration (300 μM) for all three modulators, and examined their effect on Na^+-homeostasis. We found that neither the activators nor the antagonist caused a measurable change in the intracellular Na^+ level of sebocytes.

Because of the relatively high (millimolar) intracellular Na^+ concentration, fluorescent dye-based techniques may be unable to identify small alterations in the ion concentrations. Thus, we decided to assess the TRPM5 function indirectly as well, using Fura-2 AM-based fluorescent Ca^{2+}-measurement.

Although TRPM5 itself is not permeable for Ca^{2+}, but it may exhibit some spontaneous activity at 37 °C, leading to a Na^+-dominated depolarizing background current, which can be increased and decreased by channel activators and antagonists. Hence, in an indirect functional test, we observed how this hypothetical, Na^+-dominated background current is affected by the modulation of TRPM5 and how this affects the alterations in the intracellular Ca^{2+} levels.

Since the Ca^{2+}-permeable TRPV4 channel is highly expressed and functionally active in sebocytes, we treated the cells with TRPM5 modulators (or an equal volume of vehicle) and then added an ultrapotent, selective TRPV4 agonist (GSK1016790A; 50 nM). In line with our Na^+-measurement data, we found that none of the TRPM5 modulators affected the Ca^{2+}-influx induced by the specific TRPV4 agonist GSK1016790A (Fura-2 AM).

Collectively, the completed functional assays on the ion homeostasis, together with the RT-qPCR, western blot, and siRNA transfection-mediated gene silencing efforts demonstrate that, unlike hair follicles, human sebocytes are unlikely to express TRPM5 in a functionally active form, and even if they do, the tested modulators are unable to measurably influence the activity of the channel on these cells.

The TRPM5 antagonist TPPO increases the lipogenesis in human sebocytes and influences the immune phenotype of the cells

The above data suggested that functional expression of TRPM5 on human sebocytes is unlikely; thus, modulation of hair follicles TRPM5 by truly selective

TRPM5 modulators may not cause sebaceous gland-related side effects. However, it is always possible that an agent initially thought to be specific to a particular target may later be found to be non-selective; therefore, we wanted to test whether those TRPM5 modulators that have already been shown to affect the hair cycle exert any biological effects on human sebocytes.

For that reason, our next goal was to explore the effects of the TRPM5 antagonist TPPO as well as of a selected activator. Since DMP outperformed Hept in prolonging the anagen phase in hair follicles, besides TPPO, we decided to use DMP as an agonist in most of our experiments.

As a first step, we confirmed the viability data seen in the MTT-assay using Nile Red labeling, for which we used the fluorescence intensity of the polar (membrane) lipids, which is proportional to the cell count. Nile Red measurements after 24 and 48 hours showed that, up to 300 μM, TPPO and DMP had negligible effects on the level of polar lipids, while at 1000 μM, TPPO significantly reduced it. Thus, we concluded that both TPPO and DMP can be used without risk of cytotoxicity up to a concentration of 300 μM.

To determine the effect of modulators on sebaceous lipids, we again used Nile Red labeling, but this time, we tested the levels of neutral lipids. We found that DMP had no effect, whereas TPPO significantly and concentration-dependently increased the lipid synthesis in sebocytes during 24- and 48-h treatments. This was not reversed by the TRPM5 activators, indicating that (in a perfect agreement with our expression data) the effect was TRPM5-independent.

The next question we wanted to answer regarding the biological effects of TPPO was about the effect of its effective lipogenic concentration on the immune phenotype of the sebocytes. To answer this question, we performed RT-qPCR and ELISA experiments after 3- and 24-h of TPPO-treatments. We found that TPPO significantly increased the mRNA expression of IL-6, it differentially affected the expression of IL-1α and IL-1β, and it did not significantly alter the expression of IL-8. In parallel, we also examined the effect of TPPO on the cytokine release using the supernatants of the same cultures that have been tested by RT-qPCR. We found that TPPO did not significantly influence the release of IL-6 (3- and 24-h treatments) or

IL-8 (3-h treatments), but it significantly reduced IL-8 levels in case of 24-h treatments.

Activation of Akt and EGFR is involved in mediating the lipogenic effect of TPPO

To identify the signaling pathways involved in mediating the effects of TPPO, we used phosphokinase array, which allowed us to simultaneously investigate several relevant signaling pathways.

In the experiment, the effects of 300 μM TPPO were investigated following 10, 30, and 60 min of treatment. These treatments increased the phosphorylation of several "acne-relevant" signaling molecules in a time-dependent manner, including Akt, p38α mitogen-activated protein kinase (MAPK), epidermal growth factor receptor (EGFR) and heat shock protein (HSP)-27, which also functions as a down-stream molecule of p38α MAPK.

GSK690693 (Akt), tyrphostin AG 1478 (EGFR), neflamapimod (Nefl; p38α MAPK), and J2 (HSP27) were used to inhibit these pathways. Using these inhibitors, we showed that inhibition of Akt, EGFR and p38α MAPK significantly reduced TPPO-induced lipogenesis (Nile Red labeling). When the levels of polar lipids were examined, we found that the Akt inhibitor GSK690693 and the EGFR antagonist tyrphostin AG did not affect the level of the cell count-dependent polar lipids, indicating that inhibition of Akt and EGFR most likely did indeed inhibit TPPO-induced sebaceous lipid production of the individual cells. In contrast, the p38α MAPK inhibitor Nefl significantly and concentration-dependently reduced the fluorescent signal from polar lipids, suggesting that the lipostatic effect was most likely only virtual and was actually caused by a reduction in cell number. Likewise, treatment with J2 (inhibitor of the p38α MAPK down-stream molecule HSP27) led to a significant concentration-dependent reduction in polar lipid levels as well.

As a next step in our experiments, we also investigated the role of EGFR by siRNA transfection. First, we selected the most appropriate siRNA construct and experimental design in a preliminary experiment. Next, using the said construct, we found that EGFR expression was significantly suppressed on post-transfection day 4, and, in a perfect agreement with our pharmacological data, selective gene silencing of EGFR was able to completely abolish the lipogenic effect of TPPO.

TPPO-induced IL-6 up-regulation is independent of the lipogenic Akt signaling, and is likely mediated by EGFR and p38α MAPK

Using the most potent lipostatic concentrations of inhibitors (Nefl: 1 μM, GSK690693: 10 μM, tyrphostin AG: 10 μM), we also investigated the role of the aforementioned signaling pathways in the TPPO-induced IL-6 up-regulation. We found that Nefl and tyrphostin AG were able to significantly reduce TPPO-induced IL-6 up-regulation, whereas the Akt inhibitor GSK690693 did not affect it. Our results indicate that TPPO-induced IL-6 up-regulation is likely to be independent of the lipogenic Akt signaling and may rather be the result of EGFR and p38α MAPK activation.

Results of RNA-Seq analysis suggest that TPPO significantly modulates the immune phenotype of human sebocytes and has an impact on the expression of several potentially "acne-relevant" target genes

To get a deeper insight into the mechanism of action of TPPO, SZ95 sebocytes were treated with 300 μM TPPO or vehicle as a control for 24 hours, and the collected samples were analyzed by RNA sequencing. It is important to highlight that, in agreement with our RT-qPCR results, RNA sequencing did not identify any TRPM5-specific sequences in the samples, confirming again that human sebocytes indeed do not express this channel.

Importantly, pathway analysis using the "GO: Molecular Function" pathway database of genes exhibiting significant (≥1.5-fold change, $P<0.05$) up- or down-regulation revealed that TPPO treatment modulated several "sebocyte-relevant" pathways, including "*CXCR chemokine receptor binding*", "*acylglycerol O-acyl transferase activity"*, and "*insulin-like growth factor binding*".

Since the insulin-like growth factor-1 receptor (IGF-1R) is a known positive regulator of sebaceous lipogenesis and it is a possible up-stream regulator of the Akt signaling in sebocytes, the above analysis raised the possibility that TPPO may directly or indirectly activate IGF-1R and this interaction might be responsible for the lipogenic effect. Nile Red labeling was used to address this question. Based on our results, the IGF-1R antagonist AG1024 did not affect TPPO-induced sebaceous lipid production, indicating that IGF-1R is unlikely to mediate the effects of TPPO.

Moreover, the analysis also identified several important target genes that were significantly affected by TPPO (≥1.5-fold change, $P<0.05$). On one hand, it reduced the expression of several "acne-relevant" inflammatory and other molecules, including colony-stimulating factor 2 (CSF2, also known as granulocyte-macrophage colony-stimulating factor [GM-CSF]), C-X-C motif chemokine ligand 1 (CXCL-1, also known as GRO-α), CXCL2, CXCL6, IL-32, NFKB inhibitor α (NFKBIA), tumor necrosis factor superfamily 15 member (TNFSF15), matrix metalloproteinase 9 (MMP9), and ATP-binding cassette A subfamily 1 member (ABCA1), while it increased the expression of diacylglycerol O-acyltransferase 2 (DGAT2).

Since ABCA1$^{-/-}$ mice were found to have enlarged sebaceous glands and higher free cholesterol levels in the skin, and DGAT2 is known to be a key enzyme in triglyceride synthesis, we next investigated whether activation of Akt, p38α MAPK, and EGFR pathways, respectively, played a role in the down-regulation of ABCA1 and up-regulation of DGAT2.

Our results show that TPPO was indeed able to increase the expression of DGAT2 and decrease the expression of ABCA1 over the course of 24-h treatments. Interestingly, the effect of 300 μM TPPO on ABCA1 was not affected by any of the inhibitors. In contrast, the up-regulation of DGAT2 was prevented by the Akt inhibitor GSK690693 (10 μM), whereas inhibition of p38α MAPK (Nefl; 1 μM) and EGFR (tyrphostin AG; 10 μM) did not affect it.

Thus, our data suggest that TPPO-induced lipogenesis is most likely mediated by the Akt-dependent up-regulation of DGAT2 and EGFR activation, and it may be contributed by the Akt-, p38 MAPK- and EGFR-independent down-regulation of ABCA1.

Anti-inflammatory concentration of FX inhibits proliferation of epidermal keratinocytes

In order to study proliferation, we first used the CyQUANT-assay, and treated conventional "2D" keratinocyte cultures with 14 μM FX or vehicle. Using HaCaT human immortalized keratinocytes, we found that FX-treatment inhibited cell proliferation following 48-h treatments, and this effect was reproducible in primary

keratinocytes as well. Indeed, anti-proliferative effect was also seen in NHEK cultures over the course of 48- and 72-h treatments.

As a next step, the anti-proliferative effect observed in HaCaT keratinocytes and in NHEKs was also verified in a more "*in vivo*-like" model system (3D reconstructed human epidermis equivalent). Our cultures were subjected to 48-h FX-treatment (14 μM). After sample processing, we first used Ki-67 immunolabeling, because nuclear expression of Ki-67 is a typical, widely used, and reliable marker of cell proliferation.

We found that FX decreased the ratio of Ki-67+ (proliferating) cells, but the effect was not statistically significant (P=0.0554) due to the strong donor dependence of the ratio of Ki-67+ nuclei in the control group. To overcome this issue, we continued our analysis using Fisher's exact test to assess the effects at the level of the individual donors. This analysis revealed that the effect of FX on proliferating (Ki-67+) and non-proliferating (Ki-67-) cells was significant for all three donors (P=0.0016, P<0.0001, and P=0.0001, respectively); thus, our results suggest that an effective anti-inflammatory concentration of FX (presumably through indirect inhibition of the pro-proliferative PI3K pathway) does indeed suppress proliferation of epidermal keratinocytes during long-term treatments.

The anti-inflammatory concentration of FX does not significantly affect the differentiation and barrier-forming capacity of human epidermal keratinocytes

Following the proliferation assays, we also investigated how the anti-inflammatory concentration of FX (14 μM) affects the differentiation of epidermal keratinocytes and, through this, their barrier-forming capacity.

Using the 3D model, we first examined the thickness of certain epidermal layers following FX-treatment using hematoxylin-eosin (HE) staining, and performed image analysis using images of the histological sections. Histomorphometric analysis showed that FX-treatment did not induce significant changes in the thickness of the epidermal layers.

To get deeper insight into the effects of FX, RNA sequencing was performed on preconfluent (i.e., actively proliferating) and differentiating HaCaT keratinocytes, which were treated with vehicle or FX (14 μM) in "high Ca^{2+}" medium for 24 hours after growing confluent. As shown by principal component analysis, FX had no

significant effect on the changes in gene expression patterns associated with cell differentiation, as the control and FX-treated groups were essentially indistinguishable on the basis of principal component 1, which accounted for 85.7% of the total differences between the samples.

Consistent with the above, we also found that the number of genes that were significantly (≥2 fold-change, $P<0.05$) up-regulated (46 genes) or down-regulated (38 genes) by FX was relatively low. Importantly, however, pathway analysis identified "*mitotic cell cycle process*" as a pathway significantly regulated by FX, confirming that over the course of longer treatments, potent anti-inflammatory concentration of FX may indeed shift keratinocytes towards a less proliferative phenotype. Taken together, our data suggested that the anti-inflammatory and putative anti-pruritic effects of FX may be associated with anti-proliferative activity on a longer time scale (48-72 h).

To investigate how FX affects keratinocyte differentiation, we next used primary keratinocytes to examine changes in the expression of two early differentiation markers (K1 and K10), which are widely used in experimental dermatology research. Pre-confluent (proliferating) and post-confluent cultures were prepared using cells of three NHEK donors, and the differentiating cultures were treated with vehicle or 14 μM FX in "high Ca^{2+}" solution for 24 hours. We found that FX-treatment did not reduce the differentiation-related up-regulation of K1 and K10 compared to the vehicle-treated control.

Expression of K1 and K10 have also been investigated at the protein level in the 3D model using semiquantitative immunohistomorphometry. While assessing histological sections, we found that 48-h FX-treatment did not significantly alter the expression of either keratin. This result (consistent with what has been seen previously) confirms that FX at its potent anti-inflammatory concentration (14 μM) does not significantly affect epidermal differentiation.

Epidermal differentiation and thus physicochemical barrier formation can be monitored by several other markers besides K1 and K10. One of these markers is OCLN, which contributes to the formation of the tight junction barrier in the *stratum granulosum* beneath the *stratum corneum*. By examining the expression of OCLN,

we found that FX did not reduce differentiation-induced up-regulation of the molecule in primary human epidermal keratinocytes.

As a final step of our experiments, we performed functional analyses of the barrier using two model systems. Firstly, we investigated the effect of FX on the barrier-forming capacity of HaCaT keratinocytes by real-time monitoring of the electrical impedance. Analysis of the data showed that FX-treatment did not alter the differentiation-induced elevation of the signal. Last, but not least, LY assay was also used to monitor epidermal barrier integrity in the 3D model. Our results showed that topically applied LY did not extend below the stratum corneum in either of the donors after FX-treatment, suggesting that the epidermal structure remained intact after FX administration. Taken together, our data suggest that the potent anti-inflammatory concentration of FX is unlikely to significantly affect the differentiation and barrier-forming capacity of human epidermal keratinocytes. This indicates that the anti-inflammatory and putative anti-pruritic effects of FX may be associated with anti-proliferative effects on a longer time scale (48-72 hours) without significant negative effects on differentiation and barrier formation.

Discussion

The skin acts as a complex defensive barrier, protecting the body from various external factors, harmful substances and pathogenic microorganisms. This protection is provided by physicochemical, microbiological, and immunological barriers, and by the close interaction of these barrier components. The intact structure and proper functionality of the cutaneous barrier is essential, as its damage, such as disruption of epidermal keratinocyte differentiation or sebocyte function, can contribute to the development of diseases with complex pathogenesis, such as atopic dermatitis or psoriasis.

Members of the TRP ion channel superfamily are expressed in many cell types of the skin, and they have complex regulatory functions. Sebaceous gland cells are no exception. In recent years, several TRP channels have been shown to be expressed on human sebocytes. Of these, TRPV1, TRPV3, and TRPV4, which are mainly permeable to Ca^{2+}, are negative regulators of sebaceous lipogenesis, while activation of TRPV3 also has a significant pro-inflammatory effect.

A recently published paper described that another member of the TRP family, TRPM5, may also be present in human sebaceous glands. TRPM5 is a Ca^{2+}-activated channel, but it is only permeable to monovalent cations. According to the said publication, it is an effective pro-anagen regulator of the hair cycle, and it may be a promising therapeutic target in various hair growth disorders. Due to their anatomical location, treatments targeting the TRPM5 channel in hair follicles may also affect the biology of adjacent sebaceous glands, leading to the development of unwanted side effects. Thus, our aim was to investigate the expression of TRPM5 and the impact of TRPM5 modulators in human sebocytes. The first part of this thesis summarizes the experiments that have been carried out to investigate this question.

In our experiments, we used the human immortalized SZ95 sebocytes (one of the best *in vitro* model systems for human sebaceous glands), and challenged the cells by using an antagonist (TPPO) and two activators (Hept and DMP) to modulate TRPM5.

First, using several complementary experimental methods, we have shown that TRPM5 is unlikely to be expressed in a functionally active form on human sebocytes. Indeed; although we tested two different TRPM5-specific TaqMan assays, we could

not detect mRNA expression of the channel and, in line with this, we failed to detect TRPM5-specific sequences in the samples during subsequent RNA sequencing (PRJNA1037731).

Likewise, although two different TRPM5-specific antibodies were tested in western blots, none of them resulted in specific bands at the molecular weights predicted by the manufacturers and reported in the UniProt database. Moreover, siRNA transfection-mediated gene silencing of TRPM5 did not significantly alter the optical density of the putative TRPM5-specific bands either, and none of the TRPM5-modulators induced measurable changes in the Na^+ and Ca^{2+} homeostasis of the sebocytes.

Although our above data strongly argued that the channel is probably not expressed in a functionally active form in human sebocytes, we decided to continue our experiments and to investigate whether those modulators that are thought to be TRPM5-selective induce TRPM5-independent, non-specific effects in these cells. Since DMP outperformed Hept in prolonging the anagen phase in hair follicle studies, we used DMP and TPPO in most of our subsequent experiments.

First, we continued our work by investigating lipid production, one of the most important characteristics of sebocytes. The fluorescent signal of polar lipids (correlating with cell number) confirmed what we had seen in the MTT assay: up to 300 μM, the tested modulators can be used without risk of cytotoxicity (Nile Red labeling). Moreover, assessment of neutral (sebaceous) lipids indicated that DMP had no effect on the lipogenesis up to a concentration of 1000 μM, while non-cytotoxic concentrations of TPPO dose-dependently increased cellular lipid production over the course of 24- and 48-h treatments. Furthermore, co-administration of DMP or Hept (both at 100 μM concentrations) could not reduce this effect, confirming that it was indeed TRPM5-independent.

Next, we showed that effective lipogenic concentration of TPPO (300 μM) differentially affected the cytokine profile of the sebocytes. Indeed, in RT-qPCRs performed after 3- and 24-h treatments, we observed a time-dependent effect on IL-1α and IL-1β expression, whereas TPPO up-regulated IL-6 expression and did not significantly influence IL-8 expression. Interestingly, IL-6 release also showed the same trend as expression, but in this case the difference was not statistically

significant, while IL-8 release was significantly reduced by 24-h TPPO treatment. Taken together, these data suggest that the effect of TPPO on the immune phenotype is complex. This observation was further complemented by our RNA sequencing data. Indeed, potent lipogenic concentration of TPPO significantly reduced the expression of several relevant inflammatory molecules, including CSF2 (GM-CSF), CXCL1 (GRO-α), CXCL2, CXCL6, IL-32, NFKBIA, TNFSF15, and MMP9. Since most of these effects (as well as the reduction in IL-8 release) appear to be anti-inflammatory, our data raise the possibility that, through a moderate increase in sebaceous lipid production and a predominantly anti-inflammatory effect, TPPO (or its safe functional analogues) may exert beneficial effects in diseases associated with inflammation and skin dryness.

Besides, we also aimed to explore the mechanism of the above effects. First using phosphokinase array, we tested the effective lipogenic concentration of TPPO (300 μM) in case of short-term treatments (10, 30, and 60 min). While evaluating the array, we have found that TPPO was able to modulate the phosphorylation of several relevant signaling molecules, such as Akt, EGFR, and p38α MAPK in a time-dependent manner, as well as HSP27, which may act as a down-stream effector of p38α MAPK.

Next, using selective pharmacology inhibitors selected based on the phosphokinase array, we showed that the lipogenic effect of TPPO was likely to be related to the activation of Akt and EGFR pathways, as their inhibitors (GSK690693 and tyrphostin AG) were able to reduce TPPO-induced sebaceous lipid production. Interestingly, the p38α MAPK and HSP27 inhibitors (Nefl and J2) also reduced the levels of sebaceous lipids, but their apparent lipostatic effect was most probably only virtual (i.e., occurred due to the reduction of the number of cells), and was not due to a reduction in the lipid production of the individual cells. In contrast, a purely lipostatic effect was seen when Akt and EGFR were pharmacologically blocked. The involvement of EGFR was also confirmed by siRNA transfection-mediated selective gene silencing.

Next, we investigated the role of the above signaling pathways in mediating the TPPO-induced IL-6 up-regulation. We found that, while Akt is unlikely to be involved, p38α MAPK and EGFR signaling are most likely important in the TPPO-

induced IL-6 up-regulation, as their inhibitors (Nefl and tyrphostin AG) reversed the TPPO-induced alteration in the cytokine expression.

In order to get deeper insight into the biological effects of TPPO, we performed RNA sequencing comparing the transcriptome of human sebocytes treated with vehicle or 300 μM TPPO for 24 hours. The analysis revealed that several relevant signaling pathways (e.g., "*insulin-like growth factor binding*") were significantly affected by TPPO-treatment. As IGF-1 is known to stimulate production of sebaceous lipids in sebocytes through the activation of Akt signaling, the possibility that TPPO may directly or indirectly activate IGF-1R was raised. To address this question, we assessed the effects of TPPO in the presence of the IGF-1R antagonist AG1024, and we found that IGF-1R is most likely not a target of TPPO, as the IGF-1R antagonist AG1024 did not affect its lipogenic effect.

Importantly, RNA sequencing has identified two possible regulatory molecules that could play a role in the lipogenic effect of TPPO. Indeed, TPPO was found to significantly down-regulate ABCA1 and up-regulate DGAT2. According to the literature data, these molecules appeared to be promising target genes, since $ABCA1^{-/-}$ mice exhibited larger sebaceous glands and higher free cholesterol levels in the skin, whereas DGAT2 was known to be a key enzyme in triglyceride synthesis. Thus, we next asked whether activation of Akt, p38α MAPK, or the EGFR pathway plays a role in the TPPO-induced down-regulation of ABCA1 or up-regulation of DGAT2. We found that ABCA1 down-regulation was not affected by the inhibition of these pathways, whereas DGAT2 up-regulation was specifically linked to Akt activity, as GSK690693 was able to reverse the TPPO-induced up-regulation of the expression. This also implies that, although EGFR signaling could theoretically play a role in the activation of both Akt and p38α MAPK pathways, in our case the activation of the Akt pathway was most likely independent of EGFR.

In conclusion, our data suggest that the use of truly specific TRPM5 modulators would be unlikely to induce direct sebaceous gland-related side effects, but that TPPO may have a beneficial effect on sebaceous gland biological processes in a manner independent of TRPM5. Our data raise the possibility of the use of TPPO (or its safe functional TPPO analogues) in the treatment of pathological conditions associated with inflammation and skin dryness.

In the second part of the thesis, we investigated the effects of FX, a well-known antidepressant, on the proliferation and differentiation of human epidermal keratinocytes. Our research group has recently shown that, when applied at 14 μM, FX exerted potent anti-inflammatory effects on cultured human epidermal keratinocytes and suppressed the TLR3 activator p(I:C)-induced release of the itch mediator endothelins through indirect inhibition of the PI3K pathway.

PI3K signaling is a positive regulator of epidermal keratinocyte proliferation and plays an important role in enhancing the proliferation of lesional keratinocytes in psoriasis. Since alterations in the proliferation-differentiation balance of keratinocytes can impair the epidermal barrier, and, hence, can lead to various side effects, in the present series of experiments, we aimed to investigate how effective anti-inflammatory concentration of FX (14 μM) affected the proliferation and differentiation of human epidermal keratinocytes during long-term (48- and 72-h) treatments.

As a first step, we used CyQUANT proliferation assay to investigate how 14 μM FX affected cell count of HaCaT keratinocytes. We found that following 48-h treatments, FX reduced cell proliferation, and this effect could also be reproduced using primary epidermal keratinocytes.

To confirm the translational relevance of the findings, 3D epidermis-equivalent cultures were treated with FX for 48 hours, and the ratio of proliferating cells was determined by Ki-67 labeling. Although the effect was slightly below the significance limit (P=0.0554) due to the strong donor-dependence of the ratio of Ki-67+ nuclei within the control group, we found that administration of FX resulted in a remarkable decrease in the ratio of Ki-67+ cells in all three donors. This was further confirmed by Fisher's exact test at the individual donor level. Indeed, this test showed that the effect of FX was highly significant in all three donors. Our data therefore suggested that, on a longer time scale, the anti-inflammatory and putative anti-pruritic effect of FX may be associated with anti-proliferative activity.

Next, we investigated the effects of FX on the differentiation of epidermal keratinocytes and on the barrier-forming capacity of the cells. Although analysis of hematoxylin-eosin staining in FX-treated 3D models did not reveal any changes in epidermal thickness, we wanted to get deeper insight into the effects of FX and

performed RNA sequencing. For sequencing, we analyzed RNA samples from three groups: preconfluent (i.e., actively proliferating) and postconfluent differentiating HaCaT keratinocytes treated with 14 μM FX or its vehicle in "high Ca^{2+}" medium ("Ca^{2+}-switch") for 24 hours after reaching confluence.

Principal component analysis showed that FX-treatment had no significant effect on differentiation-related changes of the gene expression patterns, as the vehicle- vs. FX-treated groups were essentially indistinguishable according to principal component 1, accounting for 85.7% of the total differences. This was also confirmed by the low number of genes that have been significantly (≥2 fold-change, $P<0.05$) up- (46 genes) or down-regulated (38 genes) by FX. Interestingly, however, complementing and indirectly confirming the previously discussed data on the proliferation, pathway analysis revealed that FX had a significant effect on "*mitotic cell cycle process*", suggesting that FX-treatment indeed affected keratinocyte proliferation.

Next, we have further investigated the mRNA expression of certain early differentiation markers (K1 and K10) using primary keratinocyte cultures of three different donors, and found that FX did not reduce the differentiation-induced up-regulation of these keratins over the course of 24-h treatments. To verify this at the protein level, we used our 3D epidermal-equivalent cultures, and analyzed the expression of K1 and K10 by semi-quantitative immunohistomorphometry. Our results showed that FX had no significant effect on the expression of these keratins over the course of 48-h treatments, and hence, FX most likely did not influence differentiation and barrier forming capacity of epidermal keratinocytes. In line with these data, using NHEKs, we have also showed that FX did not reduce the differentiation-induced up-regulation of the mRNA expression of OCLN, another important differentiation marker that plays a key role in the formation of tight junction barrier.

Finally, we intended to directly assess the effect of FX on the barrier-forming ability of keratinocytes. To this end, we performed functional studies. First, we monitored the electrical impedance of HaCaT keratinocytes. During this real time measurement, we again applied a "Ca^{2+}-switch" when the confluent state (plateau phase) was reached. Our data showed that FX did not change the differentiation-

related elevation of the signal, i.e., it probably did not impair the integrity of the intercellular connections formed during differentiation. Last, but not least, as a final step in our experiments, we performed a LY-based ("*outside-in*") dye penetration assay using 3D epidermis-equivalents. In line with the previous findings, this assay also showed that FX (14 μM) did not impair the barrier integrity of the epidermis-equivalents, as the fluorescent LY did not penetrate below the *stratum corneum* in any of the donors. Thus, we concluded that the effective anti-inflammatory concentration of FX is unlikely to impair differentiation and barrier forming capacity of human epidermal keratinocytes.

In summary, our data suggest that in addition to its previously reported anti-inflammatory and putative anti-pruritic effects mediated by the inhibition of the highly "psoriasis-relevant" PI3K pathway, FX may reduce proliferation of epidermal keratinocytes without impairing differentiation and barrier formation. These findings suggest that topical FX application may exert beneficial effects in hyperproliferative inflammatory skin diseases, such as psoriasis.

Summary

In the first part of the thesis, we investigated the expression and functional role of TRPM5 (an ion channel recently demonstrated to be expressed in human hair follicles) in human SZ95 sebocytes. While our results demonstrated that TRPM5 was not expressed on sebocytes in functionally active form, its antagonist (TPPO) was able to significantly increase sebaceous lipogenesis. This effect was mediated via Akt- and EGFR-coupled signaling pathways, through the Akt-dependent up-regulation of DGAT2. In addition, TPPO exerted differential effects on the immune phenotype of sebocytes. Indeed, it increased IL-6 expression in an EGFR- and p38α MAPK-dependent manner, and it simultaneously suppressed the release of IL-8, as well as the mRNA-level expression of several pro-inflammatory cytokines. Collectively, our data suggest that the use of specific TRPM5 modulators would be unlikely to result in direct sebaceous gland-related side effects. However, TPPO (or its safe functional analogues) may affect sebaceous gland biology in a TRPM5-independent manner.

In the second part of our experiments, we investigated the effects of the potent anti-inflammatory concentration of FX (14 μM) on the proliferation and differentiation of human epidermal keratinocytes. We found that FX exerted an anti-proliferative effect in epidermal keratinocytes, but it did not affect epidermal thickness, it did not have a significant effect on the examined differentiation markers, and it did not impair the barrier-forming capacity of the keratinocytes. In conclusion, together with our previously published findings, our current data demonstrated that dermatological application of the antidepressant FX may be possible: in addition to its anti-inflammatory activity, 14 μM of FX may exert anti-proliferative effect on human epidermal keratinocytes without detrimentally affecting their differentiation and barrier-forming capacity.

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Appendix - List of own publications

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Candidate: Dorottya Ádám-Nagy
Doctoral School: Doctoral School of Molecular Medicine
MTMT ID: 10063659

List of publications related to the dissertation

1. **Ádám, D.**, Arany, J., Tóth, K. F., Póliska, S., Váradi, J., Kolozsi, P., Tóth, D., Niehues, H., van den Bogaard, E. H., Soeberdt, M., Abels, C., Oláh, A.: Fluoxetine exerts anti-proliferative effect in human epidermal keratinocytes.
   Arch Dermatol Res. 317 (1), 1-6, 2025.
   DOI: http://dx.doi.org/10.1007/s00403-024-03711-9
   IF: 1.8 (2023)
2. **Ádám, D.**, Arany, J., Tóth, K. F., Pető, O., Nyitrai, T., Tóth, I. B., Póliska, S., Zouboulis, C. C., Oláh, A.: The TRPM5 antagonist triphenylphosphine oxide (TPPO) increases sebaceous lipogenesis and modulates immune phenotype of human sebocytes in a TRPM5-independent manner.
   Exp. Dermatol. 34 (5), 1-13, 2025.
   DOI: http://dx.doi.org/10.1111/exd.70115
   IF: 3.5 (2023)

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3. Tóth, K. F., **Ádám, D.**, Arany, J., Ramirez, Y. A., Bíró, T., Drake, J. I., O'Mahony, A., Szöllősi, A. G., Póliska, S., Kilic, A., Soeberdt, M., Abels, C., Oláh, A.: Fluoxetine exerts anti-inflammatory effects on human epidermal keratinocytes and suppresses their endothelin release.
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The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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