Methodological refinement of the psoriasis animal model and the role of Transient Receptor Potential (TRP) ion channels in psoriasiform dermatitis

PhD thesis

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INTRODUCTION

Psoriasis is a chronic, immune-mediated, polygenic inflammatory skin disease, affecting 2-3% of the general population [1]. The most common type of psoriasis, accounting for 90% of all cases, is psoriasis vulgaris [2]. Clinical manifestation of the disease is characterized by sharply demarcated, scaly, inflamed skin lesions. Plaques occur most commonly on the region of elbows, knees, back and scalp, but they can develop to be generalized throughout the body. Psoriatic skin is characterized by histopathological changes, including abnormal proliferation and differentiation of keratinocytes, dilation of blood vessels in papillary dermis, accumulation of neutrophils and a moderate T cell inflammatory infiltrate. Although psoriasis is among the most extensively studied skin disorders, the exact pathogenesis of the disease is still not known.

Aldara (5% imiquimod)-induced psoriasiform skin inflammation in mice is the most frequently used animal model to study the pathomechanism of psoriasis [3]. Imiquimod (IMQ) is a nucleotide-like small molecule, immunomodulatory effect of IMQ is mediated via TLR 7 and 8 molecule expressed by dendritic cells and macrophages [4]. Topical application of IMQ on the dorsal skin of mice results in the development of erythema, scaling, parakeratosis, acanthosis and T cell infiltration as well as it reproduces the characteristic histopathological changes of human psoriasis [5,3,6,7]. Despite its advantages (cost-effective, easy to use, the inflammatory reaction can be triggered in 5-7 days, and it is versatile: can be combined with genetically modified animals), there are several limitations. One of the major disadvantages of the model is that in addition to the topical effects, development of systemic inflammatory reaction with signs of significant weight loss, elevation of inflammatory cytokines level in blood, severe dehydration, worsened general condition and pain, which in severe cases causes untimely death of the treated mice [8 9,10,11,12].

Numerous observation proves that there is an interaction between the nervous system and immune cells in peripheral tissue, and sensory nerves play an important role of the regulation in immune processes [13]. There are several studies in the literature in which psoriasis patients with nerve damage have exhibited unilateral local improvement, even complete remission, of their psoriasis in the affected dermatomal region[14]. In case, if patients regained neural function, it was associated with a recurrence of psoriatic plaques in that area. In contrast, patients with chronic nerve damage remained clear or nearly clear of their psoriasis in the affected dermatomal region[15]. These clinical findings support the role of neurons and neurotransmitters as important mediators in the disease process of psoriasis as well as the secreted neuropeptides contributed to sustain the inflammation.

The activation of transient receptor potential (TRP) ion channels expressed on primary sensory neurons contributes to the exocytosis of neuropeptides accumulated at the nerve endings. TRP channels are nonselective cation channels that are expressed primarily in peptidergic sensory neurons

[16]. TRP receptors are polymodal, both physical (voltage, temperature, mechanical stimuli) and chemical (endogenous, exogenous) stimuli play role in their activation. In the skin TRP channels are expressed in many cell types, including sensory neurons and skin resident cells (keratinocytes, melanocytes and immune/inflammatory cells) [17]. Among these diverse cell types, TRP channels participate in physiological processes ranging from sensation to skin homeostasis. In addition, there is a growing body of evidence implicating abnormal TRP channel function, as a product of excessive or deficient channel activity in pathological skin conditions such as chronic pain and itch, dermatitis, vitiligo, alopecia, wound healing, skin carcinogenesis, and skin barrier compromise [16,18,19].

Recently, Riol-Blanco and co-workers published a study, in which they investigated the role of TRP ion channels in psoriasis. They demonstrated that the $TRPV1^+$ nociceptors are essential to the imiquimod-induced psoriasiform skin inflammation. According to their observation, selective pharmacological ablation of nociceptors resulted in diminished production of IL-23 by dermal dendritic cells (DDCs) in Aldara-induced psoriasiform skin inflammation model, thus reducing the inflammatory reaction [20].

It is well known, that 97% of $TRPA1^+$ nociceptors also express TRPV1, while 30% (100/336) of $TRPV1^+$ nociceptors express TRPA1 [21]. In the skin these receptors are expressed not only on the sensory nerve endings, but also on non-neuronal cells (keratinocytes, melanocytes and immune/inflammatory cells) [17,22,23].

AIMS

1. Methodological refinement of Aldara-induced psoriasiform dermatitis using Finn chambers

Systemic side effects can directly or indirectly influence the results of various experiments. Currently, there is no animal model investigating psoriasis, that could eliminate the development of systemic side effects. We assume that reducing the systemic side effects of the conventional model we can produce a more localized reaction, more specific to human disease. This modified model enables the more precise examination of the pathomechanism of skin inflammation which cannot be done with the classic IMQ model due to its limitations.

2. Investigation on the role of TRPA1 and TRPV1 receptors in Aldara-induced psoriasiform skin inflammation

Currently, there are a limited information about the role of TRPA1 receptor in psoriasis, however its pathogenic role is well-known in chronic itch as well as it was proved that the presence of TRPA1 on nociceptors is necessary for the development of allergic contact dermatitis. For these reasons we consider that TRPA1 receptor plays a role in pathogenesis of psoriasis. Our aim was to examine the role of TRPA1 in imiquimod-induced psoriasiform dermatitis using Finn chambers. In this study our aims were:

1. Examination of the role of TRPA1 and TRPV1 ion channels in Aldara-induced psoriasiform skin inflammation model using Finn chambers with different knockout mice strains.
2. In *in vitro* experiments to investigate if imiquimod can directly activate TRPA1 or TRPV1 ion channels in the skin.

METHODS

Animals

Experiments were performed on female TRPA1, TRPV1, TRPA1/V1 receptor gene knockout (KO, $^{-/-}$) and wild-type (WT, $^{+/+}$) or C57BL/6J mice (8–10 weeks, 20–25 g, n=30/group). The animals were bred and kept under standard pathogen-free conditions in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of at the University of Pécs, at 24–25 °C, provided with food and water *ad libitum*. All procedures were carried out according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988). All experiments were approved by the Ethics Committee on Animal Research of at the University of Pécs, in full accordance to the Ethical Codex of Animal Experiments, and a license was assigned (license number: BA 02/2000-2/2012, BA 02/2000-36/2017).

Induction of psoriasiform skin inflammation

Psoriasiform dermatitis was induced by Aldara cream (5% imiquimod, Meda Pharma, Hungary), vaseline was used as control for the first four days (Day 0, 1, 2, 3) of the 5-day experiment. The dorsal skin of mice was shaved using an electric shaver, and the remaining hairs were completely removed with depilatory cream one day prior to the first Aldara treatment. In the experiments presented in my dissertation, two experimental paradigms were used for induction of psoriasiform dermatitis. In the original protocol (OP group) – described by van der Fits and co-workers in 2009 – 62.5 mg Aldara cream (5% imiquimod) was applied on the shaved back skin of mice. In control animals, vaseline was used in the same amount [3]. In the modified protocol (MP) group, two Finn chambers (8 mm FinnChambers on Scanpor, SmartPractice, USA) were placed on the dorsal skin of same mouse, one filled with 25 mg Aldara and the other with 25 mg vaseline. In the modified protocol a special attention was paid for the treatment of the same skin areas on each day of the experiment. All experimental procedures were carried out under ketamine (100 mg/kg i.p., Richter, Hungary) and xylazine (5 mg/kg i.p., Lavet, Hungary) anaesthesia. The anesthetized animals were sacrificed using cervical dislocation at the end of the experiments (Day 4). Treated dorsal skin, spleen tissue, dorsal root ganglia (DRG) and blood samples were fixed in 6% formalin or stored at -80 $^{\circ}$C, for further analysis.

Measurement of dorsal skin thickness

Dorsal skin thickness was measured using an engineer's micrometer (Moore and Wright, Sheffield, England) with 0.1 mm accuracy, before starting the treatment (Day 0, control measurement) then prior to treatment with Aldara or control cream on the treated areas on each day of the experiments (Day 1, 2, 3, 4). Data were expressed as percent increase of back skin thickness compared with the initial values.

Measurement of blood perfusion changes

Blood perfusion was detected with LASCA (LAser Speckle Contrast Analysis - PeriCam PSI System; Perimed, Sweden) method [24, 25]. Regions of interest (ROI) were selected according to the treated area on the dorsal skin. Mean perfusion of the treated area was generated by PimSoft software (Perimed, Sweden). Data were expressed as percentage of blood perfusion change compared to the initial values (Day 0, control measurement).

Skin scaling score

The Aldara- or vaseline-treated dorsal skin area of mice were evaluated daily by three trained dermatologists who were blinded for both experimental protocols. Skin scaling was scored, ranging from 0 to 4, as represented in the following: 0 – none, 1 – slight, 2 – moderate, 3 – marked, 4 – maximum.

Measurement of spleen weight

To examine systemic side effect of Aldara treatment spleen tissue of animals were collected and weighed at the end of the experiment (Day 4). Values of the different groups were compared to each other.

Radioactive $^{45}Ca^{2+}$ uptake and Ca^{2+} influx experiments

Imiquimod-induced radioactive $^{45}Ca^{2+}$ uptake or Ca^{2+} influx was determined on HaCaT (human immortalized keratinocytes) and TRPA1 or TRPV1 receptor-expressing CHO (chinese hamster ovary) cell lines by scintillation counter or flow cytometry [26,27].

RT-qPCR measurement

6 and 48 hours after the first treatment untreated, vaseline- and Aldara-treated dorsal skin samples (n=5) were collected and mRNA expression of IL-1β, TNF-α, IL-17A, IL-23 and IL-22 cytokines were determined by RT-qPCR [28]. DRG samples were collected on Day 4, 24 hours after the last Aldara or vaseline treatment and Iba-1 mRNA expression level was evaluated.

Measurement of proinflammatory cytokines by ELISA method

The concentration of proinflammatory cytokine TNF-α, IL-1β and interferon alpha (IFN-α) was measured from the peripheral blood samples collected 6 hours following the first Aldara treatment using ELISA method.

Histology and immunohistochemistry

Aldara- and vaseline-treated skin tissue samples were formalin-fixed (6%) and embedded in paraffin, 5 µm sections were cut and stained with haematoxylin-eosin or chloroacetate esterase to determine general histological changes of inflammation. The following primary antibodies were used for immunohistochemically studies: rabbit monoclonal anti-mouse CD4 (ab183685, Abcam, Cambridge, UK; dilution 1:1000), rabbit polyclonal anti-mouse TRPV1 (ab31895, Abcam, Cambridge, UK; dilution 1:1000), rabbit polyclonal anti-mouse TRPA1 (ab68847, Abcam, Cambridge, UK; dilution 1:300), rabbit polyclonal anti-mouse Ki-67 (AB9260, EMD Millipore Corporation, Temecula, CA, USA; dilution 1:250), and rabbit polyclonal anti-mouse CD11b (NB110-89474, Novus Biologicals, CO 80112, USA; dilution 1:400).

Statistical analysis

Results were expressed as mean±standard error of the mean. Statistical analysis was conducted using GraphPad Prism 7 for Windows (GraphPad Software, USA). Probability values *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ were regarded as significant.

RESULTS

1. Methodological refinement of Aldara-induced psoriasiform dermatitis using Finn chambers

Clinical signs of psoriasis were similar to the OP and MP group after Aldara treatment

Clinical signs of psoriasis, such as erythema and scaling were consistently observed in Aldara-treated animals, however, they were not seen in vaseline-treated skin using the two different disease induction techniques. Significant increase in Aldara-treated dorsal skin blood flow and scaling was observed in OP and MP group compared to the control group or skin area, but there was no significant difference between the two different techniques. Dorsal skin thickness was significantly higher after Aldara treatment in the MP group compared to the Aldara-treated OP group on each day of the experiment.

Equivalent histopathological alterations after Aldara treatment in the OP and MP groups

Histological sections of Aldara-treated skin obtained from both experimental model clearly showed typical alterations present in psoriasis: keratinocyte hyperproliferation, hyperkeratosis, parakeratosis, Munro microabscesses as well as dilated capillaries and T cell infiltration of dermal layer. Number of

Munro microabscesses and thickness of epidermal layer were determined on the histological sections, but there were no significant differences between the Aldara-treated OP and MP groups. Distribution of Ki-67$^+$ cells was similar in the two different experimental group after Aldara treatment as well as an increasing number of CD11b$^+$ cell was observed after Aldara treatment in both MP and OP group.

The systemic inflammatory response was reduced in the MP group

After the first Aldara treatment significant weight loss was observed in the OP and MP groups compared to the vaseline-treated OP group and this difference persisted until the end of the experiment. Body weight loss was significantly less in the MP group compared to the Aldara-treated OP group after the first, second and third day of the experiment. Spleen enlargement was highest in the Aldara-treated OP group, whereas it was significantly reduced in the modified protocol using Finn chambers. There was no significant difference between spleen weight of the MP group and vaseline-treated OP group. IFN-α, IL-1β and TNF-α cytokine concentrations were determined in the peripheral blood samples of mice 6 hours after the first Aldara treatment. Concentrations were decreased in plasma samples of the MP group compared to the Aldara-treated OP group in case of all cytokines. This difference was significant in the level of IFN-α and IL-1β, but TNF-α concentration was not significantly modified. No significant difference was detected between the MP group and vaseline-treated OP group in case of any cytokines.

2. Investigation on the role of TRPA1 and TRPV1 receptor in Aldara-induced psoriasiform skin inflammation

Increased inflammatory response in the absence of TRPA1 receptor

In the first step, psoriasiform dermatitis was induced on the dorsal skin of TRPA1 wild type and knockout mice using MP protocol. Infiltration, blood perfusion and scaling of the treated skin areas were investigated on each day of the 5-day experiment. Imiquimod treatment resulted in increased skin infiltration, blood flow and scaling in both TRPA1 WT and KO mice. Interestingly, higher inflammatory activity was detected in all clinical parameters in TRPA1 KO mice compared to the wild type.

More severe histological alterations in the absence of TRPA1 receptor in psoriasform dermatitis

The typical histologic hallmarks of human psoriasis (keratinocyte hyperproliferation, parakeratosis, hyperkeratosis, Munro's microabscesses) were observed in Aldara-treated TRPA1 WT and KO mice. Histological sections were scored semiquantitatively based on three parameters: thickness of epidermal layer, number of Munro's microabscesses and number of dilated capillaries. After the evaluation of the complex histopathologic score, we found that the Aldara-induced skin inflammation is more pronounced in TRPA1 KO mice compared to the WT skin samples. In the study of inflammatory

cell infiltration, we found that the number of neutrophil granulocytes and $CD4^+$ in the dermis was elevated after Aldara treatment compared to vaseline-treated skin, but there were no significant differences between the two strains.

Increased inflammatory cytokine mRNA expression in TRPA1 KO mice

mRNA expression of IL-1β, TNF-α, IL-23, IL-17A and IL-22 cytokines were determined in TRPA1 WT and TRPA1 KO mice skin samples in different time points of the experiment (6 and 48 hours after the first Aldara treatment). A significantly increased expression of IL-1β, TNF-α, and IL-22 mRNA was detected in the TRPA1 KO group compared to the WT mice 6 hours after the first Aldara treatment, reaching maximal differences at 48 hours. IL-17A mRNA expression levels of both strains peaked at 48 hours and it was significantly higher in TRPA1 KO mice compared to TRPA1 WT mice. IL-23 mRNA expression was even more pronounced in TRPA1 WT mice as in the KO mice at 6 hours, and then higher expression levels were measured in KO mice 48 hours after the first Aldara treatment. At the end of the experiment, all of the cytokine mRNA levels were decreased to baseline values.

Reduced inflammatory response in the absence of TRPV1 receptor

In our studies, Aldara-induced psoriasiform skin inflammation was investigated in TRPV1 wild type and knockout mice. Imiquimod-induced skin thickness, blood perfusion and scaling of the treated skin were monitored on each day of the 5-day experiment. Our result showed that the level of the Aldara-induced skin inflammation was more pronounced in TRPV1 WT mice compared to TRPV1 KO mice.

Influence of TRPV1 on the role of TRPA1 in IMQ-induced skin inflammation

Psoriasiform skin inflammation was induced in TRPV1/TRPA1 double KO animals. Our results showed that double TRPA1/V1 KO led to an inhibition of skin thickness and reduced dorsal skin perfusion. These results showed a similar trend to that observed in skin inflammation induced in the TRPV1 KO mice.

Imiquimod directly activates TRPA1 ion channels

We treated TRPA1 or TRPV1 receptor-expressing CHO cell lines and HaCaT cells with imiquimod, in order to investigate its effect on TRPA1 or TRPV1 ion channels. Our measurements showed, that IMQ induced radioactive $^{45}Ca^{2+}$ uptake in TRPA1 receptor-expressing, but not in TRPV1 receptor-expressing CHO cells. In contrast, only a low level of $^{45}Ca^{2}$ uptake was detected in HaCaT cells by imiquimod. In flow cytometry experiments, we demonstrated that IMQ induced dose dependent Ca^{2+} influx in TRPA1 receptor-expressing CHO cells and this response can be inhibited by selective TRPA1 antagonists (A967079, HC030031).

Expression of TRPA1, but not TRPV1 receptors on CD4⁺ cells

Using confocal microscopy of IMQ-treated skin, we showed the presence of $CD4^+$ cells in the skin which also express TRPA1 receptors. Intriguingly, there were no TRPV1 and CD4 co-localization in skin tissue sections.

Increased Iba-1 expression level in DRG sample of TRPA1 KO mice after Aldara treatment

After Aldara treatment the relative mRNA expression level of Iba-1 macrophage activation marker was three-fold higher in DRG sample of TRPA1 KO mice compared to the DRG samples of untreated or TRPA1 WT mice on Day 4 of the experiment.

DISCUSSION

Currently, the most widely accepted animal model to investigate the pathomechanism of psoriasis is the Aldara-induced psoriasiform skin inflammation in mice. The popularity of the model is likely associated with several advantages offered to scientists: it is easy to use, it is relatively inexpensive, the inflammatory reaction can be triggered in 5-7 days, and it is versatile (can be combined with other methods, such as genetically modified animals). However, in addition to its advantages, the method has several drawbacks. There are several publications in the literature in which spleen enlargement, significant weight loss, severe dehydration and systemic inflammation (elevated inflammatory cytokines level in blood) was observed in mice treated with imiquimod [3,29,11,30]. These results clearly demonstrate that topical treatment of mice with IMQ results in systemic effects, which causes the major disadvantages of the model. Although this phenomenon is frequently overlooked or disregarded when interpreting data generated by the imiquimod model, systemic inflammation may significantly influence the results. Dehydration, for example, causes significant changes in skin structure, leading to thinner and tighter skin. Systemic and cutaneous dehydration may also severely impact skin barrier and immune functions, and thus, may substantially influence the outcome of the experiments.

Systemic symptoms are likely associated with at least two factors. First, the area of treated skin in the traditional model is considerably large, approximately 15% of the total body surface area of mice [31]. Imiquimod treatment over such a large skin area possibly leads to general symptoms, and is also observed in humans [32,33]. Secondly, the grooming behaviour of the animals results in the ingestion of imiquimod, generating type I IFN induction and activation in the gut, and consequently, leading to systemic responses.

In the first part of our work - using Finn chambers - we refined the methodology of the imiquimod-induced psoriasiform skin inflammation model developed by *van der Fits* and co-workers. This new technique proved to be sufficient to elicit skin reactions such as edema, infiltration, scaling, increased blood perfusion, and psoriasiform histopathological alterations, similar to the conventional imiquimod model. However, our new method leads to considerably reduced systemic inflammatory reactions in

the animals, as indicated by the moderate splenomegaly and weight loss, as well as little or no significant increase of IL-1β, TNF-α and IFN-α concentrations in the blood samples. We assume the decreased systemic response is due both to the smaller IMQ-treated skin area and the prevention of oral intake. A further advantage of the method is that the psoriasiform and the control skin areas are on the same animal. This fact decreases the likelihood of inter-animal differences and ensures the model is even more cost-effective. Furthermore, our novel method is ideally suitable to perform prolonged imiquimod treatment studies. Thus, it may potentially contribute to the refinement of the IMQ model, to more accurately mimic the chronic nature of psoriasis, and also prove beneficial in further studies of psoriasis comorbidities. Finally, our experimental approach may also be used in mouse model experiments with other topically applied drugs (such as TPA and acetone), to prevent the ingestion and the systemic consequences of these compounds.

In the second part of our work, we investigated the role of TRP receptors using different knockout mice (TRPV1 KO, TRPA1 KO and TRPV1/TRPA1 double KO) in our localized model (MP method) of Aldara-induced psoriasiform dermatitis.

Imiquimod-induced skin inflammation in TRPV1 KO mice was similar to the observation of Riol-Blanco and co-workers using RTX (resiniferatoxin)-treated animals. Based on their findings the presence of TRPV1 receptors on the peripheral nociceptors is essential for the IMQ-induced psoriasis. However, we have shown in *in vitro* experiments that this could not be a direct effect of IMQ via the TRPV1 receptor as IMQ does not activate TRPV1 expressing CHO cells. Hence, we propose that TRPV1 receptor is activated by an as yet unknown mediator released by other cells (e.g., keratinocytes) in response to IMQ. In contrast, additional elimination of the TRPA1 channels in the TRPV1/TRPA1 double KO mice did not modify the outcome of the IMQ-induced pathology, further supporting the dominant role of TRPV1 in the process.

Surprisingly, in our experiments TRPA1 KO mice showed an enhanced Th1-type immune response, an increase in skin thickness and blood flow, and more severe psoriasis symptoms after Aldara treatment compared to the WT animals. It suggests that under normal circumstances TRPA1 downregulates the inflammatory process. In our experiments, we have provided evidence that the expression of Iba-1 (macrophage activation marker) mRNA is significantly elevated after IMQ treatment in TRPA1 KO mice compared with WT animals. These results suggesting that neuronal expression of TRPA1 ion channel can regulate the imiquimod-induced nerve activity.

An increasing number of publication report that TRPV1 and TRPA1 channels can form a heterodimer in sensory neurons during basal conditions, and both are able to cross-regulate each other's activity (cross-sensitization/desensitization) during inflammation [34]. Fischer and co-workers described that the presence of TRPA1 exerts a functional inhibition of TRPV1 [35]. This suggests that TRPA1 likely acts by modulating neuronal excitability, possibly via the regulation of TRPV1 activity. Although the

proinflammatory effects of TRPA1 activation are well established, there is emerging evidence for its protective effects in inflammatory processes. Recently, in a mouse model of wound healing, pharmacological activation of $TRPA1^+$ nociceptor in the skin was described to reduce scar formation and can also promote tissue regeneration [36]. Capsazepine, originally classified as a TRPV1 antagonist, has been shown to protect the development of experimental colitis via TRPA1 agonism[37]. In various colitis animal models it was shown that TRPA1 deletion enhanced inflammatory responses [38,39].

It is well known, that the main mechanism of action for IMQ is considered to be via TLR7 on cutaneous macrophages and dendritic cells in mice [4]. However, it is not only able to exert its effect via TLR7, but also induces inflammasome activation via NALP3 signalling pathway [40,41]. Here we present the first evidence that IMQ has TRPA1 agonist activity. In our *in vitro* experiments we have shown that imiquimod induced dose-dependent Ca^{2+} influx in TRPA1-transfected CHO cells and this response was selectively inhibited by the TRPA1 antagonists (A967079 and HC030031). By comparison, IMQ did not induce Ca^{2+} influx in CHO cells transfected with recombinant TRPV1, indicating that it is unlikely that IMQ would directly influence the function of $TRPV1^+$ nociceptors in the skin. Thus, IMQ as a TRPA1 agonist, may potentially activate neural and non-neural cells, and exert anti-inflammatory activity in the skin.

Inflammatory mediators are known to regulate TRPV1 activity via a range of pathways, including specific G-protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs) [34]. KC-Tie2 murine model of psoriasisform skin disease characterized by the increased presence of cutaneous nerves in mouse skin. Cutaneous denervation in the KC-Tie2 psoriasisform mouse model also results in improvement of acanthosis and decreases in dendritic cell number, in IL-23 protein expression and in T cells infiltrate. Thus, it resulted in reduced inflammatory reaction. This effect was suggested to be mediated by nerve-derived substance P and calcitonin gene-related peptide [42]. Potentially, IMQ, similar to capsazepine, may directly activate TRPA1 on sensory neurons, leading to the partial desensitization of these nociceptors to TRP-mediated stimuli in psoriasis. This, in turn, may result in a reduced release of neuropeptides from nerve endings, leading to attenuated dendritic cell activation, decreased IL-12 or IL-23 release, and diminished subsequent generation of T helper 1 or T helper 17 cells, and cutaneous inflammation. We have a growing knowledge of the anti-inflammatory role of TRPA1-mediated desensitization of nociceptors in different inflammatory diseases, so applying already known or new “TRPA1 desensitizers” may provide new therapeutic options in the future.

In summary, our results enlightened new aspects of neuro-immune interaction in the skin. It is assumed, that the activation of different TRP ion channels influences the immune response in the tissue in different ways, for this reason TRP channels play an important role in tissue-specific immune response. Specific activation or inhibition of TRP channels may be a new target in the treatment of immune-mediated skin diseases.

SUMMARY OF THE NEW FINDINGS

1. We refined the imiquimod-induced psoriasiform dermatitis model in mice using Finn chambers. Our localized method reproduces psoriatic skin alterations with considerably reduced systemic inflammatory reactions. Possessing psoriasiform and control skin areas on the same mouse also reduces inter-individual differences. Furthermore, our novel method is ideally suitable to perform prolonged imiquimod treatment studies. The new method may potentially contribute to more accurately mimic the chronic nature of psoriasis, and also prove to be beneficial in further studies of psoriasis comorbidities.
2. These results are the first to show that TRPA1 has a protective role in IMQ-induced psoriasiform dermatitis.
3. We provided first evidence that IMQ is a potent TRPA1 agonist and can directly activate cells expressing TRPA1, but not TRPV1.
4. We provided further evidence that the genetic (KO mice) or pharmacologic (using RTX desensitization) ablation of TRPV1 receptors decrease the Aldara-induced psoriasiform dermatitis. It is assumed that the presence of $TRPV1^+$ nociceptor is necessary for the development of imiquimod-induced skin inflammation.

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PUBLICATIONS

Publications related to the thesis:

1. **Horváth S**, Komlódi R, Perkecz A, Pintér E, Gyulai R, Kemény Á. Methodological refinement of Aldara-induced psoriasiform dermatitis model in mice. *Sci. Rep.* **9**,3685 (2019). (D1) **IF:3.998**

2. Kemény Á*, Kodji X*, **Horváth S***, Komlódi R, Szőke É, Sándor Z, Perkecz A, Gyömörei C, Sétáló G, Kelemen B, Bíró T, Tóth BI, Brain SD, Pintér E, Gyulai R. TRPA1 acts in a protective manner in imiquimod-induced psoriasiform dermatitis in mice, *J. Invest. Dermatol*. **138,** 1774–1784 (2018). (D1) **IF:6.29**

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2. Kuczmog, A., Galambos, A., **Horváth, Sz**., Mátai, A., Kozma, P., Szegedi, E., Putnoky, P. (2012) Mapping of crown gall resistance locus *Rcg*1 in grapevine. *Theor Appl Genet* **125**:1565–1574. (Q1) **IF:3.658**

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1. Á. Kemény, **S. Horváth**, R. Komlódi, A. Perkecz, C. Gyömörei, E. Pintér, R. Gyulai: The TRPA1 ion channel mediates inhibitory effects on imiquimod-induced psoriasiform skin inflammation. *Journal of Investigative Dermatology* 136: 9S. p.S228. (2016). 46th Annual Meeting of the European Society for Dermatological Research. München, Németország: 2016.09.07 - 2016.09.10. (poster)
2. Á. Kemény, **S. Horváth**, R. Komlódi, X. Kodji, Z. Sándor, É. Szőke, A. Perkecz, E. Pintér, R. Gyulai: Opposing effect of TRPA1 and TRPV1 on imiquimod-induced psoriasiform dermatitis. *Journal of Investigative Dermatology* 137: 10S. Supplement 2, p.S251. (2017). 47th Annual Meeting of the European Society for Dermatological Research. Salzburg, Ausztria: 2017.09.27-30. (poster)
3. **S. Horváth**, Á. Kemény, R. Komlódi, A. Perkecz, C. Gyömörei, E. Pintér, R. Gyulai: Methodological improvement of imiquimod-induced psoriasiform dermatitis model. *Journal of Investigative Dermatology* 137: 10S. Supplement 2, p.S272. (2017). 47th Annual Meeting of the European Society for Dermatological Research. Salzburg, Ausztria: 2017.09.27-30. (poster)
4. **Horváth S**, Kemény Á, Komlódi R, Perkecz A, Pintér E, Gyulai R. LB1561 Localized Aldara-induced psoriasiform dermatitis model using Finn chambers. International Investigative Dermatology Conference. Orlando, Florida 2018. 05. 16-19. poster
5. Kemeny A, **Horváth S**, Komlódi R, Kodji X, Sándor Z, Szoke É, Perkecz A, Pinter E, Gyulai R. LB1563 Protective role of TRPA1 on imiquimod-induced psoriasiform dermatitis. International Investigative Dermatology Conference. Orlando, Florida 2018. 05. 16-19. poster
6. Kemény Ágnes, **Horváth Szabina**, Gyömörei Csaba, Botz Bálint, Bölcskei Kata, Pintér Erika, Gyulai Rolland: A TRPV1 és TRPA1 receptorok szerepe az imiquimoddal kiváltott egér pszoriázis modellben. A Magyar Kísérletes és Klinikai Farmakológiai Társaság Experimentális Farmakológiai Szekciójának IX. Szimpoziuma, Velence, Magyarország. 2015.03.26-28. (poster)
7. **Horváth Szabina**, Kemény Ágnes, Komlódi Rita, Gyömörei Csaba, Bölcskei Kata, Pintér Erika, Gyulai Rolland (2015): TRPA1 szerepének vizsgálata imiquimod-indukálta psoriasiform

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8. Ágnes Kemény, **Szabina Horváth**, Rita Komlódi, Csaba Gyömörei, Kata Bölcskei, Erika Pintér, Rolland Gyulai: Investigation of the role of TRPA1 receptors in imiquimod-induced psoriasiform skin inflammation in mice. 23rd Leuven TRP meeting. Leuven, Belgium: 16-18 Sept 2015. (poster)
9. Kemény Ágnes, **Horváth Szabina**, Pintér Erika, Gyulai Rolland: Imiquimoddal indukált pszoriázis modell vizsgálata egérben. IV. Harkányi Psoriasis Konferencia 2015.10.16-17. (lecture)
10. **Horváth Szabina,** Kemény Ágnes, Komlódi Rita, Gyömörei Csaba, Bölcskei Kata, Pintér Erika, Gyulai Rolland (2015): TRPA1 szerepének vizsgálata imiquimod-indukálta psoriasiform bőrgyulladásban. Magyar Dermatológiai Társulat 88. Nagygyűlése, Budapest, Magyarország. (lecture)
11. **Horváth Szabina**, Kemény Ágnes, Komlódi Rita, Gyömörei Csaba, Pintér Erika, Gyulai Rolland: A TRP ioncsatornák szerepe a bőr immunfolyamatainak szabályozásában (2016). Magyar Dermatológiai Társulat 89. Nagygyűlése. Budapest, Magyarország. 2016. 11. 24-26. (lecture)
12. Kemény Á., **Horváth Sz**., Komlódi R., Gyömörei Cs., Bölcskei K., Pintér E., Gyulai R.: A Tranziens Receptor Potenciál Ankyrin 1 receptorok szerepe az imiquimoddal kiváltott pszoriázis-szerű egérmodellben. Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája (2016), Pécs, Magyarország. Program összefoglalók P3.114. (poster)
13. **Szabina Horváth**, Ágnes Kemény, Rita Komlódi, Anikó Perkecz, Csaba Gyömörei, Erika Pintér, Rolland Gyulai: Imiquimod-induced psoriasiform skin inflammation is enhanced in transient receptor potential ankyrin-1 ion channel knockout mice (2016). 3rd Meeting of Middle-European Societies for Immunology and Allergology. Budapest, Magyarország. 2016. 12. 01-03. (poster)
14. Kemény Ágnes, **Horváth Szabina**, Komlódi Rita, Gyömörei Csaba, Szőke Éva, Sándor Zoltán, Pintér Erika, Gyulai Rolland: A TRPA1 és TRPV1 ioncsatornák szerepének vizsgálata az imiquimoddal kiváltott pszoriázis-szerű gyulladás modellben. A Magyar Élettani Társaság, a Magyar Kísérletes és Klinikai Farmakológiai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság közös Vándorgyűlése, Debrecen, Magyarország. 2017. 06. 13-16. (lecture)
15. **Horváth Szabina**, Kemény Ágnes, Komlódi Rita, Szőke Éva, Sándor Zoltán, Gyömörei Csaba, Pintér Erika, Gyulai Rolland: TRPA1 receptorok gyulladáscsökkentő hatása Aldara-indukált psoriasiform bőrgyulladásban. Doktoranduszok a klinikai kutatásokban. Pécs, Magyarország. 2017. 10.28. (lecture)
16. **Horváth Szabina**, Kemény Ágnes, Komlódi Rita, Szőke Éva, Sándor Zoltán, Gyömörei Csaba, Pintér Erika, Gyulai Rolland: A Tranziens Receptor Potenciál Ankyrin 1 (TRPA1) receptorok gyulladáscsökkentő hatása Aldara-indukált psoriasiform bőrgyulladásban. Magyar Dermatológiai Társulat 90. Nagygyűlése. Budapest, Magyarország. 2017. 11. 23-25. (lecture)
17. Kemény Ágnes, **Horváth Szabina**, Komlódi Rita, Perkecz Anikó, Pintér Erika, Gyulai Rolland: Az imiquimoddal kiváltott psoriasiform bőrgyulladás állatkísérletes modelljének továbbfejlesztése. Magyar Dermatológiai Társulat 90. Nagygyűlése. Budapest, Magyarország. 2017. 11. 23-25. (poster)
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19. **Horváth Szabina**, Komlódi Rita, Perkecz Anikó, Pintér Erika, Gyulai Rolland, Kemény Ágnes: Methodological refinement of Aldara-induced psoriasiform dermatitis model using Finn chambers. Magyar Dermatológiai Társulat 91. Nagygyűlése. Budapest, Magyarország. 2018. 11. 29-12.01. (lecture)

20. Kemény Ágnes, **Horváth Szabina**, Komlódi Rita, Perkecz Anikó, Pintér Erika, Gyulai Rolland: A TRPV1 és a TRPA1 ioncsatornák szerepének összehasonlítása az imiquimoddal kiváltott psoriasiform bőrgyulladás állatkísérletes modelljében. Magyar Dermatológiai Társulat 91. Nagygyűlése. Budapest, Magyarország. 2018. 11. 29- 12. 01. (poster)

List of presentations, not related to the thesis:

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