Novel pharmaceutical approaches for the promotion of dermal wound healing

Summary of the PhD Thesis

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Introduction

The skin represents the largest organ of the human body, which functions as the first line of protection from a range of external stress factors that can lead to injury. Restoration of skin integrity is a fundamental process that ensures survival. The repair response is a complex, dynamic interplay of multiple cell types. This process is divided into three overlapping phases: inflammation, proliferation, and remodeling.

Impairment of the wound healing process can lead to chronic wounds, which is defined as a barrier defect that has not healed in 6 weeks. The most common types are diabetic foot ulcers, venous or arterial leg ulcers and pressure ulcers. Chronic wounds represent a significant source of morbidity, with more than 6 million people suffering in the U.S. alone and expenditures of ~$9.7 billion annually.

Diabetes impairs each phase of wound healing. Diabetic wounds exhibit a persistent inflammatory phase, due to altered neutrophil and monocyte phenotypes, abnormalities in the expression and activity of cytokines and growth factors, the proliferation phase is characterized by endothelial cell dysfunction, impaired angiogenesis, decreased keratinocyte migration and proliferation, resulting in inadequate granulation tissue formation and reduction in wound tensile strength. Although, there has been exponential growth of research in the field of wound management over the past years, with standard of care, still only 50% of patients with diabetic foot ulcers heal, and to date, no single therapeutic agent has been successful in improving the healing rate above 50– 60%.

Immediately after injury several damage signals are released, such as Ca^{2+} waves and hydrogen peroxide (H_2O_2) gradients. As a major secondary messenger, intracellular Ca^{2+} is involved in various intracellular signaling pathways e.g., excitation-contraction coupling. The main intracellular Ca^{2+} stores are the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) and the mitochondrion. There are two major receptors regulating the Ca^{2+} release from the SR/ER, the inositol 1,4,5-triphosphate receptors (IP3Rs) and the ryanodine receptors (RyRs). In mammalian tissues, three genes encode three RyR isoforms and many types of cells express each of them. RyR1 (skeletal muscle type) and RyR2 (cardiac type) are primarily expressed in the skeletal and the cardiac muscle and they are pivotal for excitation–contraction coupling, whereas RyR3 (brain type) contributes to the intracellular calcium regulation in the brain**.** Recently, the functional existence of RyR in epidermal keratinocytes has been demonstrated.

Intracellular Ca^{2+} signaling in keratinocytes is essential for cellular processes, including migration, proliferation, differentiation, barrier homeostasis and release of proinflammatory

cytokines. It has been previously shown that activation of excitatory receptors, such as N-methyl-D-aspartate receptor (NMDA), nicotinic acetylcholine receptor, P2X purinergic receptor, and RyR induces elevation of intracellular calcium concentration and delays barrier recovery of the skin. On the other hand, the inhibition of calcium channels, such as voltage-gated calcium channel, P2X receptor, and RyR accelerate barrier recovery. However, no information is available concerning the effects of RyRs on the healing of full-thickness dermal wounds.

In addition to the above-mentioned receptors, serotonin receptors (5HTRs) and serotonin reuptake transporters (SERT), which are responsible for the uptake of serotonin from the extracellular space to the intracellular space, decreasing the extracellular serotonin concentrations, are also widely expressed on many tissues, including cells present within the skin. Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter and cytokine. Although it is mostly synthesized in the gastrointestinal mucosa and the central and peripheral nervous system, the biosynthetic pathways of serotonin have also been found in cells playing key roles in all phases of wound healing such as inflammatory cells (neutrophil granulocytes, macrophages, T lymphocytes), cutaneous human fibroblasts and keratinocytes. There has been growing evidence that serotonin has an important role in the promotion of cutaneous wound healing. The importance of 5-HT in hemostasis has been well-established. In the inflammatory phase platelets release 5-HT, which enhances the recruitment of neutrophil cells and macrophages. While in the early stages, pro-inflammatory M1 macrophages predominate, later the number of anti-inflammatory M2 macrophages increase contributing to the resolution of inflammatory responses. 5-HT modulates macrophage functions by skewing macrophages towards an anti-inflammatory M2 phenotype. Interestingly, 5-HT has been known to promote fibroblast proliferation in vitro, and also promotes myofibroblast differentiation, which suggests the important role of the serotoninergic pathway in wound healing. Prior studies have shown that selective serotonin reuptake inhibitors (SSRIs), for example fluoxetine (FLX) have anti-inflammatory effects such as suppression of T cell activation, cytokine secretion and proliferation. The underlying mechanism is likely due to that FLX suppresses intracellular Ca^{2+} signaling by decreasing IP3- and RyR mediated Ca^{2+} release. Therefore, examining SSRI local effects on chronic wounds, characterized by stalled inflammatory phase is reasonable.

Aims

Our principal goal was to study the effects of locally applied drugs in the process of wound healing. For this aim, *in vitro* and *in vivo* experiments were designed. The entire study was divided into 2 parts (mentioned as Study I and Study II, respectively).

In Study I, the major objectives were:

- to examine the effect of induction and inhibition of RyRs on full-thickness wounds in SKH-1 mice,
- to evaluate the rate of wound closure by means of photographic imaging and histological analysis,
- to examine the effects of 4-chloro-m-cresol (4-CMC), a RyR agonist and dantrolene (DA), a RyR antagonist on keratinocyte proliferation and to monitor different parameters of the microcirculation in the wound edges by means of intravital videomicroscopy and laser Doppler flowmetry,
- to study the effects of the topical agents (4-CMC and DA) on the inflammation process of the healing,
- to monitor the effect of induction and inhibition of RyRs on wound closure of HaCaT cells with scratch test.

The goals of Study II were:

- to examine the effect of FLX as a topically applied drug on wound healing in db/db diabetic mice, a model for impaired wound healing,
- to evaluate the effect of FLX on keratinocyte migration using an in vitro scratch assay,
- to monitor if topical FLX treatment induces psychological effects, behavioral experiments on wounded diabetic mice treated with topically applied FLX were performed.

Methods

Materials

In study I, a ryanodine receptor (RyR) agonist, 4-chloro-m-cresol (4-CMC, 0.5 mM), or a RyR antagonist, dantrolene sodium salt (DA, 100 μM) was applied on the wounds of the animals. Immortalized human keratinocytes from the HaCaT-cell line after scratching were treated with 4-CMC (0.3 mM) or DA (45 μM).

In study II, full-thickness excisional wounds in db/db diabetic mice were treated with either 0.02% fluoxetine (FLX) or 2% serotonin (5-HT) dissolved in 5% w/v polyethylene glycol (5% PEG). Neonatal human keratinocytes (NHKs) isolated from human foreskin after scratching were treated with different concentrations of 5-HT (1 μmol/L 5-HT, 10 μmol/L 5-HT, 100 nmol/L 5-HT) in keratinocyte growth medium (KGM).

Animals

In study I, the experiments were performed on 12-15-week-old male SKH-1 hairless mice (body weight: 36-44 g).

In study II, the genetically diabetic male mice (db/db; BKS.Cgm+/+ *Leprdb*) were used for the experiments.

Study design

Study I.

The mice were divided into 3 treatment groups: (1) wounds were treated with sterile saline; (2) wounds were treated with 4-CMC (0.5 mM); (3) wounds were treated with DA (100 μM). Photographs were taken every 4 days (4, 8, 12, 16 and 20), then the animals were euthanized, and tissue samples were taken for histological analysis.

Monitoring of the microcirculation with intravital videomicroscopy (IVM) was performed on days 4, 8 and 12. In a separate group of mice laser Doppler flowmetry was performed on wounds treated with either 4-CMC or DA.

Xanthine-oxidoreductase (XOR) and myeloperoxidase (MPO) activities were measured during the inflammatory phase on days 1 and 4. 6 mice were assigned to each group and time point.

This part of our investigation also involved *in vitro* wound-healing assays.

Study II.

Diabetic (db/db) mice received two full-thickness 8-mm splinted circular excisional wounds. The mice were divided into 3 treatment groups: (1) wounds were treated with topically applied FLX; (2) wounds were treated with serotonin; (3) wounds were treated with vehicle control 5% w/v polyethylene glycol (PEG). Daily treatments were applied topically. On day 9, behavioral

experiments were performed. The time mice spent in light versus dark chambers was quantified, and percent time not spent in the light chamber is a measure of anxiety. Exploration ratio was used to evaluate cognitive ability in mice. On day 10, the animals were euthanized, wound tissue was fixed, sectioned, and stained for hematoxylin-eosin or immunohistochemistry. In this part of our investigation *in vitro* wound-healing assays were performed as well.

Measurement of wound area (Study I.)

Photographs were taken of the wounds with a camera. The area of the wound was measured and referred to the area determined on day 0 in order to calculate the rate of wound closure.

Intravital videomicroscopy (IVM) (Study I.)

The microcirculation was visualized with a fluorescence intravital videomicroscope. The following parameters were examined: the red blood cell velocity (RBCV, μm/s) was measured in the capillaries of wound edges. Vessel diameter (VD, μm) was assessed by measuring of all vessels in the given fields or view except those of less than 6 μm.

Microcirculatory measurements by means of laser Doppler flowmetry (Study I.)

A non-invasive laser Doppler tissue flowmeter was used to evaluate the cutaneous microvascular blood flow. A standard pencil probe producing laser beam was placed on the surface of the wound edge. We formed circular wounds as big as the probe head. We measured the flow 24 h after the surgery. First, we measured the baseline flow, and then the wounds were treated. 10 minutes later we repeated the measurements. The signal was registered for 20 seconds.

Tissue XOR and MPO activity (Study I.)

The activity of XOR, a marker of ROS production and that of MPO, a marker of tissue leukocyte infiltration, were measured from homogenized skin biopsies.

Routine histology and immunohistochemistry (Study I.)

The wound was excised, and fixed. One slide was stained with haematoxylin-eosin (H&E), while the other was used for immunohistochemical detection of Ki-67 positive cells.

In the H&E-stained sections, we measured the diameter of the wound and the length of the growing epithelial tissue on both sides. The sum of the growing epithelial tissue was referred to the initial diameter of the wound. In the Ki-67-stained slides, to analyze the epidermal proliferation in response to the treatments, we calculated the epidermal proliferation index; the amount of Ki-67-expressing basal keratinocytes were divided by the whole number of basal

keratinocytes, to determine the percentage of proliferating cells as an indicator for proliferative activity.

Cell culture and scratch test (Study I.)

Human HaCaT keratinocytes, were cultured in DMEM. For the experiments, cells were seeded into 24-well plates. 3 different treatments were applied by using 6 samples for each case.
Scratch wounding was performed with a cell scraper of 4 mm width. The cells were treated once daily with either 4-CMC (0.3 mM), or DA (45 μM), while the control group was left untreated. The entire area of a well was imaged at 24 h, 48 h, and 72 h post-wounding. DermAssess© software was used to measure the width of the scratch.

Cell culture and scratch test (Study II.)

Keratinocyte Growth: Human keratinocytes were isolated from neonatal foreskins.
Scratch Assay: Three different strains of keratinocytes were grown to confluence in KGM. The cells were either untreated (control) or treated with different concentrations of 5-HT (1 μmol/L 5-HT, 10 μmol/L 5-HT, 100 nmol/L 5-HT) in KGM at time 0. A sterile pipette tip was used to scratch three 500-μm-wide to 1-mm-wide wounds around the center of the dish. The rate of healing scratch wounds made in confluent NHK cultures was examined.

Splinted wound model (Study II.)

Two donut-shaped splints fabricated from a 1.6-mm thick silicone sheet were placed bilaterally at the designated locations and adhered with cyanoacrylate adhesive, plus eight interrupted sutures. A full-thickness wound was created within the circumference of each splint by using an 8-mm sterile skin biopsy punch. A trimmed plastic cover slip was placed on top of the splint. Daily treatments as indicated were applied topically. Day 10 wound tissue was fixed, sectioned, and stained for hematoxylin-eosin or immunohistochemistry.

Results

Study I.

Inhibition of RyRs accelerates wound closure in vivo

Planimetric analysis of the wound area on digital images showed a continuous increase in epithelialization with approximately 20% wound coverage on day 4, 50% on day 8, and 80% on day 12 in the group treated with DA. At the end of the experiment, on day 20, all of the calcium antagonist treated wounds achieved a complete wound closure, while the 4-CMC treated animals did not.

The macroscopic finding of increased rate of wound closure in the group treated with DA was confirmed by routine histology. The growing epithelial tongues of the edges of the wounds were found significantly longer on days 4 and 8, compared to the control animals. From day 12 to 20, no significant difference was found between the groups.

To determine whether the accelerated wound closure can be attributed to increased proliferation, the proliferative activity of the epidermis was quantified by analyzing Ki-67-stained sections. The epidermal proliferation index was calculated on days 4, 8, 12, 16 and 20 but our results did not show significant difference temporally or spatially between the groups.

Wound closure of HaCaT cells is accelerated by dantrolene

We investigated the effect of DA and 4-CMC on wound closure in HaCaT cell monolayers. The experimental results showed that the scratch closure occurred at a significantly faster rate in the presence of DA compared to the control, and the scratch area was completely closed after 72-hour culture. In contrast, in cultures treated with 4-CMC the gap closure was delayed by 72 h.

Dantrolene elevates the vessel diameter and the red blood cell velocity

The analysis of the IVM video records revealed that the vessel diameters did not display a change within the 4-CMC and the control groups during the observation period, while the calcium channel antagonist increased the vessel diameters by 25% on day 4 compared to the control group. This significant difference was also observed on day 8 (17%) and on day 12 (22%), as well.

It has also been shown that inhibition of the RyRs increased the red blood cell velocity in the capillaries at all times of measurements by approximately 25%, while there was no difference between the 4-CMC and the control group. The findings of laser Doppler flowmetry have confirmed the data obtained from IVM. The flow curves demonstrated consistent significant

increases in the blood flow from baseline levels to posttreatment levels with an average of 15-fold increase in the group treated with DA.

Inhibition of RyRs decreases XOR activity thereby diminishing ROS production, while does not affect MPO activity and leukocyte accumulation

MPO activity, a commonly used index of inflammatory cell accumulation, was measured during the inflammatory phase of wound healing on the first and the fourth days post-wounding. According to our results, no significant difference was found between the groups. In contrast, significant reductions of XOR activity, a critical source of ROS production, were observed in the group treated with DA on days 1 and 4 as compared with the control group, while 4-CMC did not alter the enzyme activity.

Study II.

Wound closure of NHK cells is accelerated by fluoxetine in the presence of serotonin

Although human keratinocytes have been shown to express tryptophan hydroxylase gene, an enzyme in the rate-limiting step in 5-HT synthesis, we did not find any 5-HT produced by keratinocytes above our lower limit of detection of 9.8 nmol/L. Thus, endogenous generation of 5-HT by keratinocytes may be too low to enhance migratory speed in FLX-treated keratinocytes in the absence of exogenous 5-HT. In the presence of 5-HT, however, FLX improved healing in treated cultures: 60.6% in the 10 nmol/L treatment group, 62.0% in the 100 nmol/L treatment group ($P = 0.01$), and 67.0% healed wound area in the 1 μmol/L FLX group ($P = 0.001$), relative to the 52.2% healing in the control cultures. To provide further evidence that FLX is working through 5-HT-dependent pathways, scratch wound assays were repeated in the presence of the HTR2A blocker ketanserin (KET). The HTR2A blocker KET reversed the effects of FLX on wound healing in vitro, again returning wound healing to the level of the untreated control group ($P = 0.948$). These data demonstrate not only that FLX increases keratinocyte migration in vitro but that this is dependent upon 5-HT signaling through HTR**.**

Fluoxetine promotes re-epithelialization in vivo

To test whether FLX promotes re-epithelialization in vivo, full-thickness excisional wounds in db/db diabetic mice, a model for impaired wound healing, were treated with topically applied FLX, 5-HT, or vehicle control 5% w/v polyethylene glycol (PEG). Wounds from mice treated with either 0.02% FLX or 2% 5-HT dissolved in 5% PEG showed less exudate compared with vehicle control counterparts at day 10 postwounding. Moreover, re-epithelialization was increased from an average of 39.6% in PEG-treated mice to 66.2% in mice treated with 0.2%

FLX (P = 0.01). Since the topical application of 5-HT did not result in statistically significant improvement in re-epithelialization, likely due to the short half-life of serotonin, we did not further investigate its direct effects.

Limited systemic effects of topically applied fluoxetine.

For clinical translation of a topically administered drug, ideally, systemic absorption should be minimized to limit the side effect profile. After 10 days of daily dosing with topically applied 0.2% FLX, the levels of FLX in mouse plasma ranged from 23 to 64 ng/mL, with no change in plasma serotonin concentrations. The FLX levels measured are twofold lower than plasma levels in patients treated with oral FLX at therapeutic doses and are also significantly lower than levels in mice treated with neurologically therapeutic doses of FLX, either orally or intraperitoneally administered. To further query if our topical FLX treatment induces psychological effects, we performed behavioral experiments on wounded diabetic mice treated with topically applied FLX and found that the animals treated with FLX did not exhibit significant changes in their behavior in the light/dark chamber box test, a measure of anxiety, or in the novel object recognition test, a measure of cognition. These findings indicate the potential of topically delivered FLX, contrasted to other delivery methods for improving healing with minimal systemic effects.

Discussion

Wounds of different type may considerably decrease the health-related quality of life and place substantial burden on healthcare system. Thus, there seems to be a need for novel therapeutic approaches accelerating the healing process. In our studies, we investigated the effect of already marketed drugs - DA, a muscle-relaxant and FLX, an antidepressant - on wound healing. Our study has revealed that DA, an inhibitor of RyRs, promotes macroscopic wound closure *in vivo* and the histological examination has confirmed that this agent contributes to the process of epithelialization. Furthermore, the *in vitro* experiments have shown faster closure of the keratinocyte layer after application of DA. Regeneration of the epithelium requires tightly regulated spatiotemporal process of proliferation, migration and differentiation. Calcium signals seem to have a role in these processes. Epidermis displays a characteristic calcium gradient, with low calcium levels in the lower, basal, and spinous epidermal layers, and increasing calcium levels towards the stratum granulosum that contributes to keratinocyte differentiation. It has also been described that extracellular calcium triggers an increase in the level of intracellular free calcium which subsequently promotes cell differentiation. Since epidermal injuries disturb the calcium gradient and RyRs are known to be major mediators of calcium-induced calcium release, it seemed to be presumptive that influence of these receptors may affect wound healing. Denda *et al.,* have shown that activation of RyRs delays the barrier regeneration while inhibition of RyRs by means of topical DA accelerates the barrier recovery. In the mentioned study, the injury was confined to the uppermost layer of the skin. A novelty of our investigation is the demonstration of the efficacy of DA in full thickness dermal wounds. According to our findings, inhibition of RyR contributes to the healing process via different ways. By means of immunostaining for Ki-67, we have not found significantly higher proliferation rate after application of DA. Thus, it can be assumed that increased cell migration can be responsible for the accelerated wound closure. Migration may be regulated by calcium dependent processes, but this question requires further investigation. However, our *in vivo* experiments have identified another important factor playing role in the regeneration.

The results obtained by means of IVM have shown that local application of DA led to a considerable increase in RBCV in the capillaries of the wound edge. This elevation may originate in the vasodilation of the arterioles and the relaxation of the precapillary sphincters. Measurement of vessel diameters has proven the vasodilation and the laser Doppler flowmetry has also confirmed the elevated blood flow after inhibition of RyR. It is known that RyRs are expressed in vessels of different calibres in several organs, e.g. renal resistance arterioles,

mesenteric arteries, cremaster arterioles, large cerebral arteries and in cerebral microcirculation, as well. RyRs play a pivotal role in the regulation of vascular tone but their effect may be diverse in different organs. Our results have demonstrated that DA considerably elevates the vessel diameter and the RBCV. The role of RyRs in the dermal microcirculation has not been known before. The increased perfusion of the wound area may thus result in a better oxygen and nutrient supply hereby contributing to a faster regeneration.

The present study has also revealed that DA has an impact on inflammation accompanying wounds. Inflammation is known to be the first phase of wound repair and plays an important role in healing. However, an excessive inflammatory reaction may lead to chronic wound and contribute to scar formation. Inflammation can be characterized with different factors e.g., inflammatory cell accumulation and production of reactive oxygen species (ROS). MPO, which is a lysosomal protein highly expressed in neutrophil granulocytes and macrophages, is a critical element of oxygen-dependent antimicrobial system in granulocytes and can be used as a marker of inflammatory cell accumulation. XOR is a major source of ROS in macrophages, it can also be detected in keratinocytes and it is an important component of innate inflammatory signaling. During normal healing process, the expression of XOR is upregulated shortly after wounding. Although local application of DA has not influenced the leukocyte accumulation, it considerably moderated the ROS production. According to the literature, calcium seems to play a role in the regulation of ROS forming mechanisms. Barrier injury is followed by the release of various neurotransmitters from the epidermis e.g., ATP, dopamine, and glutamate (Glut). After wounding, Glut achieves high concentrations in the skin. Accumulation of Glut stimulates NMDA receptors which increase the intracellular calcium level triggering a ryanodine-gated calcium release from ER. Moreover, inhibition of NMDA receptors or RyRs suppresses ROS production in astrocytes. The calcium influx may also lead to mitochondrial calcium overload which can enhance mitochondrial superoxide generation. The mentioned processes seem to be self-propelling, because ROS-induced damage in the mitochondria leads to XO activation and further ROS production. Furthermore, exposure to ROS also activates the RyRs. It can be assumed that restraining of intracellular calcium release by inhibition of RyRs results in a decrease of ROS formation.

The potential anti-inflammatory effect of DA has already been suggested by previous studies. In animal experiments, DA was found to suppress the production of pro-inflammatory cytokines (TNF-α, IL-12 and IFN-γ), to increase the quantity of anti-inflammatory cytokines (IL-10), to attenuate mitochondrial dysfunction and to improve survival in a murine model of

endotoxemia. On the other hand, activation of the RyRs with 4-CMC has not influenced the studied parameters, thus seems to have no effect on wound healing.

FLX has also been shown to exert anti-inflammatory and immunosuppressive effects. In a lipopolysaccharide-induced murine model of septic shock, FLX was found to reduce the expression of TNF-α and to improve survival. Moreover, FLX has been found to reduce LPS-induced ROS/RNS generation in microglial cells and also in Huh7.5 cells stimulated with hepatitis C virus respectively. Interestingly, a research on human T lymphocytes showed that FLX interferes with Ca^{2+} signaling by depleting Ca-stores, therefore leaving less Ca^{2+} available for release after IP3R or RyR activation, suggesting a possible mechanism behind the anti-infalmmatory effect of FLX. The mentioned study also demonstrated that the depletion of Ca-stores is not 5-HT mediated. In accordance with previous studies, our analysis of wound beds by quantitative RT-PCR showed a decrease in TNF, IFN-γ, and IL-6 transcript, respectively, compared with the control group, indicating that FLX decreased inflammation in the wound bed (data not shown). In addition, our study revealed that FLX shifted the local immune milieu toward a less inflammatory phenotype in vivo by promoting the generation of pro-reparative M2 macrophages at the local wound environment (data not shown). These results are in consistent with the study by F. Su *et al*., that showed that FLX can inhibit M1 activation and improve M2 activation of microglia cells, the brain-specific macrophages.

The present study has also revealed that signaling through serotonin pathways 5-HT in combination with FLX, improved keratinocyte migration *in vitro*. Furthermore, the *in vivo* impaired wound healing model has shown increased re-epithelialization after application of FLX.

According to our results topically applied FLX may interact with multiple pathways involved in wound healing.

In conclusion, our results have demonstrated that inhibition of calcium-induced calcium release by means of locally applied DA accelerates wound closure *in vivo* and *in vitro*. Moreover, DA increases the blood flow of the skin. We have also shown that inhibition of RyRs decreases XOR activity thereby diminishes ROS production. While there are a variety of materials available for wound care, such as dexpanthenol, sodium hyaluronate or zinc hyaluronate, which can promote wound healing by increasing fibroblast proliferation and accelerating re-epithelialization, to our knowledge there are no other agents for topical use which can additionally promote wound healing by increasing perfusion of the wound area.

In the diabetic mouse model of impaired wound healing, we have demonstrated that topical FLX improves wound healing through local effects on multiple cell types within the wound. The beneficial effects of FLX in our study appear to be independent of psychological changes. Our work has demonstrated the potential of repurposing DA, as a RyR antagonist, and FLX, an SSRI, as safe and promising therapeutic tools in order to promote dermal wound healing via different pathways.

Summary and new findings

Our in vivo investigation and in vitro experiments were focused on unraveling the roles of RyRs and 5HTRs in the process of wound healing.

We have revealed that inhibition of RyRs has beneficial effects on wound healing via action on multiple targets:

- DA accelerates wound closure in vivo
- wound closure of HaCaT cells is accelerated by DA
- DA elevates the vessel diameter and the red blood cell velocity
- inhibition of RyRs decreases XOR activity thereby diminishing ROS production

We demonstrated that FLX as a topically applied drug:

- accelerates the wound closure of NHK cells in the presence of 5-HT
- FLX promotes re-epithelialization in vivo
- topically applied FLX has limited systemic effects

Hence, these topically applied drugs represent a safe alternative for the challenging clinical problem of chronic, nonhealing wounds.

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List of publications

Full papers included in this thesis

I. Degovics D, Hartmann P, Németh IB, Árva-Nagy N, Kaszonyi E, Szél E, Strifler G, Bende B, Krenács L, Kemény L, Erős G.: A novel target for the promotion of dermal wound healing: Ryanodine receptors.

Toxicol Appl Pharmacol. 2019;366:17-24. doi: 10.1016/j.taap.2019.01.021.
IF: 3.347

II. Nguyen CM, Tartar DM, Bagood MD, So M, Nguyen AV, Gallegos A, Fregoso D, Serrano J, Nguyen D, **Degovics D**, Adams A, Harouni B, Fuentes JJ, Gareau MG, Crawford RW, Soulika AM, Isseroff RR.: Topical Fluoxetine as a Novel Therapeutic That Improves Wound Healing in Diabetic Mice.

Diabetes. 2019;68(7):1499-1507. doi: 10.2337/db18-1146.
IF: 7.720

Abstracts related to the subject of the dissertation

I. Degovics D, Hartmann P, Németh IB, Árva-Nagy N, Kemény L, Erős G: A rianodin receptorok szerepe a sebgyógyulásban. Bőrgyógy Vener Szle 89(6):p.165, 2013. (A Magyar Dermatológiai Társulat 86. Nagygyűlése, Budapest, 2013. december 12-14.)

II. Degovics D, Hartmann P, Németh I, Árva-Nagy N, Kemény L, Erős G: The effect of ryanodine receptors on dermal wound healing. Journal of Investigative Dermatology 134 (Suppl 2):S102, 2014

III. Degovics D, Hartmann P, Németh IB, Árva-Nagy N, Kemény L, Erős G: The role of ryanodine receptors in wound healing. European Surgical Research 52:178, 2014.

Other full papers

I. Szél E, Danis J, Sőrés E, Tóth D, Korponyai Cs, **Degovics D**, Prorok J, Acsai K, Dikstein S, Kemény L, Erős G: Protective effects of glycerol and xylitol in keratinocytes exposed to hyperosmotic stress.

Clin Cosmet Investig Dermatol. 2019;12:323-331. doi: 10.2147/CCID.S197946
IF: 1.970

II. Szél E, Polyánka H, Szabó K, Hartmann P, **Degovics D**, Balázs B, Németh IB, Korponyai C, Csányi E, Kaszaki J, Dikstein S, Nagy K, Kemény L, Erős G: Anti-irritant and anti-inflammatory effects of glycerol and xylitol in sodium lauryl sulphate-induced acute irritation.

J Eur Acad Dermatol Venereol. 2015;29(12):2333-41. doi: 10.1111/jdv.13225
IF: 3.029

III. Szolnoky Gy, Erős G., **Degovics D**., Németh I., Kui R., Paschali E, Vasas J, Korom E, Varga M, Kemény L: Novel aspects of lymphedema-, and venous leg ulcer-related pathomechanism and therapy
Bőr és Vener Szemle. 2019;95(2):65–68. doi 10.7188/bvsz.2019.95.2.6

Other abstracts

I. Degovics D, Erős G, Hartmann P, Gati K, Nemeth IB, Czobel M, Korponyai C, Nagy K, Kemeny L. The effects of oxygen- and hydroxyproline-containing solutions on the healing of acute wounds. Clinical Hemorheology and Microcirculation 2013; 54(2):193-194.

II. Szél E, Erős G, Hartmann P, Németh IB, **Degovics D**, Korponyai C, Dikstein S, Boros M, Nagy K, Kemény L: Anti-irritant and anti-inflammatory effects of polyols in irritant contact dermatitis. Clinical Hemorheology and Microcirculation 54(2):205, 2013.

III. Szél E, Erős G, Hartmann P, **Degovics D**, Korponyai C, Boros M, Dikstein S, Nagy K, Kemény L: Poliolok antiirritáns és gyulladáscsökkentő hatása irritatív kontakt dermatitiszben. Bőrgyógy Vener Szle 89(6):p.179, 2013. (A Magyar Dermatológiai Társulat 86. Nagygyűlése, Budapest, 2013. december 12-14.)

IV. Szél E, Erős G, Hartmann P, Németh I, **Degovics D**, Korponyai C, Kaszaki J, Polyánka H, Szabó K, Dikstein S, Nagy K, Kemény L: Poliolok antiirritáns és gyulladáscsökkentő hatása irritatív kontakt dermatitiszben. Bőrgyógy Vener Szle 90(6):p.261, 2014. (A Magyar Dermatológiai Társulat 87. Nagygyűlése, Budapest, 2014. november 27-29.)

Patent applications

I. Erős, G; Degovics, D; Hartmann, P; Kemény, L: Pharmaceutical composition comprising a ryanodine receptor antagonist for facilitating wound healing
US20160303085A1, Benyújtás éve (szabadalom): 2016, Benyújtás száma: 15/103,374, Benyújtás országa: Amerikai Egyesült Államok

II. Degovics, D; Erős, G; Hartmann, P; Kemény, L: Pharmaceutical composition comprising dantrolene analogues for treating skin wounds
HU1300720A2, Benyújtás éve (szabadalom): 2013, Benyújtás száma: P1300720, NSZO: A61K 31/4178, A61P 17/02, Ügyszám: P1300720, Benyújtás országa: Magyarország

III. Erős, G; Degovics, D; Hartmann, P; Kemény, L: Pharmaceutical compositions for facilitating wound healing
WO2015087097A2, Benyújtás éve (szabadalom): 2014, Benyújtás száma: PCT/HU2014/000124, NSZO: A61K 31/4178

IV. Erős, G; Degovics, D; Hartmann, P; Kemény, L: Pharmaceutical composition comprising a ryanodine receptor antagonist for facilitating wound healing
Benyújtás éve (szabadalom): 2016, Benyújtás száma: 201647023609, Ügyszám: 201647023609, Benyújtás országa: India

V. Erős, G; Degovics, D; Hartmann, P; Kemény, L: Pharmaceutical composition comprising a ryanodine receptor antagonist for facilitating wound healing
Benyújtás éve (szabadalom): 2016, Benyújtás száma: 201480075392.6, Ügyszám: 201480075392.6, Benyújtás országa: Kína

VI. Erős, G; Degovics, D; Hartmann, P; Kemény, L: Pharmaceutical composition comprising a ryanodine receptor antagonist for facilitating wound healing
Benyújtás éve (szabadalom): 2016, Benyújtás száma: 14837102.4, Ügyszám: 14837102.4, Benyújtás országa: Hollandia

VII. Erős, G; Degovics, D; Hartmann, P; Kemény, L: Pharmaceutical composition comprising a ryanodine receptor antagonist for facilitating wound healing
Benyújtás éve (szabadalom): 2017, Benyújtás száma: 17103927.3, Ügyszám: 17103927.3, Benyújtás országa: Hongkong