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Experimental research regarding the anti-psoriasis effect of some categories of substances in topical applications

PHD THESIS SUMMARY

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Experimental research regarding the anti-psoriasis effect of some categories of substances in topical applications - summary

1.1. **Introduction**

Psoriasis is a chronic dermatological disease, with an inflammatory and autoimmune component, which occurs because of a combination of predisposing genetic factors and external or internal triggering factors.[1] Being one of the most common chronic pathologies in dermatological practice, it is at the same time a continuous challenge in terms of treatment, with the aim of improving the quality of life of patients and increasing treatment compliance. In localized and moderate forms of psoriasis, topical treatment is instituted as a single or adjuvant therapy in combination with systemic therapy.

Topical treatment of psoriasis is currently limited to a few classes of medicinal substances including dermatocorticoids, vitamin D analogues, retinoids, calcineurin inhibitors, substances with a keratolytic role such as salicylic and lactic acid. The main line of topical treatment is represented by dermatocorticoids, substances from the class of steroid anti-inflammatories, which have demonstrated a favorable effect in this pathology, but with the disadvantage of producing adverse reactions, both local (cutaneous atrophy, local infections, tachyphylaxis) and systemic adverse reactions through the inhibition hypothalamic-pituitary-adrenal cortical axis.[2]. Interest in recent years has been focused on the discovery of new substances with anti-psoriasis potential in both topical and systemic therapy. Numerous studies have had as their central subject the research of the effect of new substances in psoriatic pathology or the improvement of existing pharmaceutical forms for certain already approved drugs. Regarding the effect of celecoxib and diclofenac in topical applications for the treatment of psoriasis, no data are available.

Following the review of literature of recent years regarding the topical therapy of psoriasis, it was concluded that the subject is of great interest and it has been proven that experimental research is still essential for the study of new substances with potential anti-psoriasis effect or for the discovery of new mechanisms of action involved in this regard, but also for improving some known pharmaceutical formulas already used in order to increase absorption at the site of action or compliance.

Between 27.06.2019 and 09.07.2020, we carried out a systematic review of literature, which aimed to research the scientific interest in this field and identify new tested substances with anti-psoriasis effect, substances already known with anti-psoriasis effect but in new

formulas (pharmaceutical form, dose, concentration), experimental model used, and mechanism of action involved.[3]

Interest in the topical treatment of psoriasis followed an upward trend during the 1951–2020 period, with 6052 articles published on the topic, of which 4572 in the period 1995–2020.[3]

Regarding research in the field of topical therapy of psoriasis, the current trend is to discover new substances with potential anti-psoriasis effect and to improve the pharmaceutical forms of substances already approved but also to research various associations between two or more substances with anti-psoriasis effect.[3]

Substances already known to have an anti-psoriasis effect like: ciclosporin, mometasone furoate in apazomal gel, calcipotriol emulsion, methotrexate in combination with salicylic acid, tazarotene with cineole 1% have been tested.[3]

Recent experimental research on animal models in the field of topical anti-psoriasis therapy includes substances with new mechanisms and pathways of action but also already known substances with anti-psoriasis effect in improved topical formulas: lycopene, salvianolic acid, albendazole, antisense microRNA, thymoquinone in ethosomal vesicles, sea buckthorn (*Hippophae rhamnoides*) oil, methotrexate with salicylic acid in ethosomal gel, nitidine, methotrexate in deformable liposomes 0.05%, 0.1%, extract of *Melissa officinalis spp. Altissima*, BMX -010, protein kinase p38 as therapeutic target with BIRB796 as inhibitor, cineole-enriched tazarotene in nanovesicles.[3]

Psoriasis mice models offer the advantage of *in vivo* study of the pathological process but also of a potential anti-psoriasis effect of some substances. The main *in vivo* psoriasis models used in non-clinical research are the psoriasis tail model and the imiquimod model. Because psoriasis is a multifactorial disease that does not occur naturally in mice, experimental models use as evaluation parameters different aspects of the disease such as clinical examination corresponding to the PASI score, histopathological examination, cytokine profile, etc.

The tail model for psoriasis was first used in 1964 by Jarett and Spearman and is based on the induction of orthokeratosis in those parts of the adult mouse tail that normally show parakeratotic differentiation.[4] [5] As an indicator of orthokeratosis, the thickness of the granular layer (which is absent or minimal in parakeratosis) is used, given that

hypogranulosis is practically the signature of the existence of psoriasis. This model has been used to demonstrate the anti-psoriasis effect of several topically applied substances. The model involves the use of male mice with an average weight of 25-30 grams. The model runs for 10 days and consists of the local administration of the substance to be investigated, daily, for two hours, in occlusion, on the proximal part of the tail. At the end of the experiment, the animals are sacrificed after general anesthesia according to the ethical norms regarding research on the laboratory animal, the tails are harvested, and the histopathological examination of the parts is performed. Thus, the presence of the granular layer and the changes in the epidermal thickness are investigated. The granular layer is evaluated qualitatively but also quantitatively by relating the length of the granular layer to the total length of the scale, between two hair follicles. Epidermal thickness is measured from the dermo-epidermal junction to the lower portion of the stratum corneum.

1.2. Working hypothesis and general objectives

The working hypothesis used in the experiment is that diclofenac and celecoxib, substances from non-steroidal anti-inflammatory drug class, have an anti-psoriasis effect. The thickness of the granular layer (which is absent or minimal in parakeratosis) is used as an indicator of orthokeratosis. The model is mainly based on morphometry, being reproducible and sensitive in the quantitative evaluation of the anti-psoriasis effect of the substances to be investigated on the epidermal differentiation process, clearly affected in psoriasis.

The aim of the study

The aim of the study is to establish, by using some experimental models to evaluate psoriasis in mice, the anti-psoriasis effect of some categories of substances such as some non-steroidal anti-inflammatory drugs (diclofenac and celecoxib).

Objectives

1. Setting up and validating the experimental method for evaluating psoriasis in laboratory animals, using the tail model.

2. The histopathological evaluation of the substances effect on the tail, in hematoxylin-eosin staining.

3. Establishing the possible mechanism by which these substances determine the anti-psoriasis effect.

1.3. General methodology

Material and method

Animals

Adult male mice weighing approximately 25 grams were used. The mice were housed, one mouse per cage with ad libitum access to water and food throughout the experiment. Also, the environmental conditions were constant throughout the experiment (light, temperature, humidity).

The approval of the ethics commission was obtained (authorization number 13110/27.10.2021) for non-clinical studies carried out on laboratory animals according to law no. 43/2014 on the protection of animals used for scientific purposes, with subsequent additions and Directive 86/609/EEC of 24 November 1986 on the approximation of laws and administrative acts of the Member States regarding the protection of animals used for experimental purposes and for other scientific purposes.

Substances

The substances used in the experiment were purchased from a producer of pure substances (Fagron, Bucharest).

The experimental study

The experimental study was carried out over 14 days as follows:

The animals were divided into groups:

Group 1: positive control group (tretinoin 0.05% in ointment base) – 6 animals

Group 2: negative control group 1 (ointment base) – 6 animals

Group 3: negative control group 2 (untreated mice) – 6 animals

Group 4: test 1 – test substance 1 in ointment base – 6 animals

Group 5: test 2 – test substance 2 in ointment base – 6 animals

Mice were treated with 0.1 ml of ointment applied topically on the proximal part of the tail for two hours, with adhesive tape fixation of a plastic cylinder in direct contact. At the end of the two hours, the cylinders were removed, and the tails were washed with warm water. This procedure was performed once a day, 5 days a week, for 2 consecutive weeks. 6

mice per group were used. Mice were weighed every two days. Two hours after the last treatment, the mice were sacrificed after general anesthesia according to the ethics of laboratory animal research, and the tails were histologically prepared (fixation in 10% formalin). Hematoxylin-eosin-stained sections of approximately 4 μm thickness were made.

The specimens obtained from the experiment were analysed histometrically for:

A) The length of the fully developed granular layer within a scale (10 scales per animal, 6 animals per group, 60 measurements per group were analysed).

B) The scale length, established between two hair follicles including the sebaceous gland (10 scales per animal were analysed, 6 animals for each group, 60 measurements per group).

C) Epidermal thickness measured between the dermo-epidermal junction and the bottom of the stratum corneum (5 measurements per scale, 10 scales per animal, 6 animals per group, 300 measurements in total were analysed).

From these data (A-C) the following derived parameters were calculated according to Bosman et al.:

D) The degree of orthokeratosis of the individual scale expressed as a percentage ratio between A) divided by B) and

E) Intensity of substance activity (drug activity) = (Okstest – Okcontrol)/(100-OKcontrol) * 100.

OK= orthokeratosis as the average of the parameters expressed as the average of the parameters obtained at point D) for the test substance (stest) respectively for the negative control group 1 – ointment base

F) Mean epidermal thickness of the group calculated as the arithmetic mean of the mean epidermal thickness of each mouse within the group.

From these calculations the following 3 parameters are calculated to evaluate the effect of the substance:

1) The orthokeratosis degree.

2) The percentual drug activity.

3) The average epidermal thickness.

For statistical comparisons, the Kruskal-Wallis test is used with the level of significance set at $p \leq 0.05$.

Data are presented as mean values.

The images were processed in the ZEN Blue program. Dimensions were calculated in micrometers.

2. Study 1- Evaluation of the anti-psoriasis effect of diclofenac using the mice tail model

The non-steroidal anti-inflammatory drug class has recently been used in the topical treatment of actinic keratoses, lesions that have the potential to transform into squamous cell carcinoma, since by inhibiting the cyclo-oxygenase 2 pathway, keratinocyte proliferation is inhibited. [6,7] [8]

Recent studies have demonstrated the involvement of diclofenac in cellular apoptosis control of keratinocytes with features of dysplasia.[9] [10]

Diclofenac is approved for the topical treatment of actinic keratoses.[11] In the clinicaltrials.gov database until April 1, 2022, no clinical trials were identified regarding the topical effect of diclofenac in psoriasis. The mechanism of action involved in the treatment of actinic keratoses is represented by the inhibition of COX-2 (cyclooxygenase 2), as this enzyme is involved in aberrant keratinocyte proliferation but also in neoangiogenesis.

Two negative control groups are used, represented by the group of untreated mice and the group of mice treated with petroleum jelly, which is used as an ointment base. The experiment was carried out in two stages. In a first stage, topically applied diclofenac was tested in concentrations of 1% and 2%. Because the results were satisfactory, it was decided to test higher concentrations of diclofenac of 4% and 8%, with negative and positive control groups, with the same experimental protocol.[12]

2.1. Results in the first part of the experiment

The degree of orthokeratosis is in the following order: tretinoin 0.05% > diclofenac 1% > diclofenac 2%.

Mean epidermal thickness is in the following order: tretinoin 0.05% > ointment base > diclofenac 1% > diclofenac 2% > untreated mice group.

The intensity of drug action is in the following order: tretinoin 0.05% > diclofenac 1% > diclofenac 2%.

There is statistical difference between negative control (untreated mice, ointment base group), positive control (tretinoin 0.05%) and diclofenac (1% and 2%) groups regarding the degree of orthokeratosis. The negative control groups (untreated mice, ointment base) differed statistically significantly from the positive control group (tretinoin 0.05%) but also from the groups with diclofenac (1% and 2%). There is no statistical difference between the positive control (tretinoin 0.05%) and diclofenac (1% and 2%) groups.

2.2. Results in the second part of the experiment

The degree of orthokeratosis is statistically significantly increased for the higher concentrations of diclofenac (4% and 8%) compared to the positive control (tretinoin 0.05%). The highest value of the degree of orthokeratosis was for diclofenac 4%.

The mean epidermal thickness is in the following descending order: tretinoin 0.05% > white soft paraffin > diclofenac 4% > diclofenac 8% > untreated mice group.

2.3. Conclusions

The tail model used in this experiment with two negative control groups (untreated mice and the ointment base – white soft paraffin) and the positive control group (tretinoin 0.05%) was validated by the fact that in the negative control group with white soft paraffin the degree of orthokeratosis was not statistically significantly different from the group of untreated mice and in the positive control group, with tretinoin 0.05%, the degree of orthokeratosis was significantly increased compared to the negative control groups.

Diclofenac 4% can be used with optimal results in terms of the degree of orthokeratosis (71.3%), higher drug activity (66.16%), with a lower risk of adverse reactions by using a lower concentration.

3. Study 2 - Evaluation of the anti-psoriasis effect of celecoxib by the mice tail model

Celecoxib belongs to the class of non-steroidal anti-inflammatory drugs, being a selective inhibitor of cyclo-oxygenase 2, with a lower risk of producing gastrointestinal adverse reactions, used in the symptomatic treatment of various rheumatological pathologies. Celecoxib has been FDA approved since 1998 for its utility in the symptomatic

treatment of rheumatoid arthritis and osteoarthritis. Although the risk of gastrointestinal bleeding is lower compared to non-selective cyclooxygenase inhibitors, it exists especially in people at increased risk of gastrointestinal bleeding. In addition, celecoxib also acts through other mechanisms such as binding to cadherin 11, considered a factor in the progression of tumor proliferation, inhibiting carbonic anhydrase 2 and 3, with additional anti-neoplastic properties. [13,14] Intestinal absorption of celecoxib is very good with risk of liver and kidney toxicity. Studies have recently appeared on the effect of celecoxib applied topically in various dermatoses with an inflammatory or autoimmune component.

The experiment was carried out in two stages. In the first stage, topically applied celecoxib was tested in concentrations of 1% and 2%. Because the results were satisfactory, it was decided to test higher concentrations of celecoxib of 4% and 8% with the negative and positive control groups, using the same experimental protocol. [12,15]

3.1. Results obtained in the first part of the experiment

Induction of keratinocyte differentiation as measured by the degree of orthokeratosis is in the following order: celecoxib 2% > tretinoin 0.05% > celecoxib 1% > white soft paraffin > untreated mice.

Mean epidermal thickness is in the following order: tretinoin 0.05% > celecoxib 2% > white soft paraffin > celecoxib 1% > untreated mice.

The percentual drug activity is in the following order: celecoxib 2% > tretinoin 0.05% > celecoxib 1%.

There is no statistical difference between the celecoxib 1% and 2% groups and the positive control group in the degree of orthokeratosis.

3.2. Results obtained in the second part of the experiment

The highest degree of orthokeratosis was for celecoxib 8%, followed by celecoxib 4%, celecoxib 2% and tretinoin 0.05%.

Mean epidermal thickness is in the following order: celecoxib 8% > tretinoin 0,05% > celecoxib 4% > white soft paraffin > celecoxib 2% > untreated mice.

The percentual drug activity is in the following order: celecoxib 8% > celecoxib 2% > tretinoin 0,05%.

The orthokeratosis degree was significantly greater for celecoxib 2%, 4% and 8% when compared to tretinoin 0,05%. The values obtained for celecoxib 8% and celecoxib 4% are very close and not statistically different. This fact shows a possible significant anti-psoriasis effect for celecoxib 2%, 4% and 8%.

Celecoxib 4% and 8% statistically significantly increased mean epidermal thickness compared to the untreated mice group. The highest mean epidermal thickness values were for celecoxib 8% followed closely by tretinoin 0.05%. Celecoxib 8% had the strongest effect in inducing the degree of orthokeratosis, followed closely by celecoxib 4%. All concentrations of celecoxib had percentual drug activity values above 50%, with a maximum for celecoxib 8% (70.59%) and a minimum for celecoxib 2% (52.16%).

3.3. Conclusions

Celecoxib 4% and 8% increased the degree of orthokeratosis statistically significantly compared to celecoxib 2%. These results suggest that both celecoxib 4% ointment and celecoxib 8% ointment can be used with a statistically similar effect.

It can be considered that celecoxib 4% can be used preferentially because it contains a lower concentration of active substance and therefore a lower risk of systemic or local side effects.

4. Study 3 – Evaluation of the anti-psoriasis effect of the combination of salicylic acid with celecoxib using the mice tail model

Salicylic acid is a compound with topical application approved for the treatment of various pathologies characterized by the impairment of cell differentiation and proliferation such as ichthyoses, psoriasis or seborrheic dermatitis. Salicylic acid irreversibly inhibits cyclooxygenases (COX-1 and COX-2) thereby stopping the conversion of arachidonic acid to prostaglandins and thromboxane. Its usefulness in rheumatic diseases is due to its anti-inflammatory and analgesic effect. Topical salicylic acid formulas treat cell hyperproliferation characterized by hyperkeratosis, as occurs in acne, warts, psoriasis, keratosis pilaris, etc.

The effect of salicylic acid when applied topically is keratolytic and antiseptic. At lower concentrations, the effect is keratoplastic, by stopping abnormal keratinization, while at higher concentrations, over 1%, salicylic acid causes keratolysis, probably because of the low ph that leads to the destruction of the stratum corneum, with desquamation. Due to its

keratolytic effect, salicylic acid is used in combination with topical substances from other drug classes such as glucocorticoids, as it can facilitate the access of the active substance to the site of action by removing scales from the psoriatic plaque. Also, during periods of clinical improvement of psoriasis, salicylic acid is successfully used with the limitation of the use of topical glucocorticoids.[16]

Salicylic acid is used for the synthesis of acetylsalicylic acid, which is administered orally, one of the most widely used pharmaceutical products. In the form of esters, amides and salts, it is the raw material for many pharmaceutical products. [17]

If salicylic acid is administered over large areas of the skin, it can be absorbed systemically and induce salicism, especially in the young child or newborn.

Celecoxib 1% applied topically in hydrogel has shown benefit when applied topically in patients with hand-foot syndrome after chemotherapy.[18]

Recent in vivo studies using the TPO inflammation model have shown the potential utility of topically applied celecoxib in conditions such as atopic dermatitis, eczema and psoriasis, in that celecoxib reduced inflammation and the level of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), hyperproliferation and neutrophilic infiltration. [19,20]

Celecoxib is a selective COX-2 inhibitor that demonstrated satisfactory anti-proliferative properties in the previous study, so we wanted to check if there is a drug potentiation effect between salicylic acid and celecoxib.

The three test groups used in the study were salicylic acid 2%, celecoxib 2% and the combination of salicylic acid 2% with celecoxib 2%, mixed in the ointment base, with negative and positive control groups, using the same experimental protocol.

4.1. Results

The degree of orthokeratosis is in the following order: celecoxib 2% and salicylic acid 2% > celecoxib 2% > tretinoin 0.05% > salicylic acid 2% > white soft paraffin > untreated mice.

The mean epidermal thickness is in the following order: tretinoin 0.05% > salicylic acid 2% > white soft paraffin > celecoxib 2% and salicylic acid 2% > celecoxib 2% > untreated mice.

The percentual drug activity is in the following order: celecoxib 2% and salicylic acid 2% > celecoxib 2% > tretinoin 0.05% > salicylic acid 2%.

Tretinoin 0.05% and salicylic acid 2% increase the degree of orthokeratosis similarly, with no statistical difference. Celecoxib 2% increases the degree of orthokeratosis either as monotherapy or in combination with salicylic acid, statistically significantly compared to all other groups tested. The degree of orthokeratosis is much higher when salicylic acid is associated (72.21% versus 59.43%). From these data it can be concluded that there is a drug summation effect between salicylic acid and celecoxib.

5. General conclusions and personal contributions

5.1. General conclusions

Premises of the paper

The research topic is represented by highlighting the anti-psoriasis effect of some substances from the NSAID class, namely diclofenac and celecoxib, through an experimental mouse model. For this purpose, the tail model was used, a classic *in vivo* model based on morphometry, valid, which can allow the study of the effect of a substance on the epidermis in general as well as the granular layer in particular. It is thus possible to evaluate the induction of epidermal differentiation as a marker of the anti-psoriatic action as well as the intensity of action of a substance.

The experimental protocol consists of the local application of the test substance, mixed in the ointment base, at the level of the proximal part of the tail in the amount of 0.1 milliliters of ointment, in occlusion, for two hours. The procedure was repeated 5 days a week for two weeks. At the end of the experiment, after ethically euthanizing the animals, the tails were harvested and fixed in 10% formalin and specifically prepared for histopathological evaluation. Mice were divided into negative control, positive control and test groups, six mice for each group.

The tail model for psoriasis is an *in vivo* model for researching the effect on epidermal differentiation of some topically applied substances, by calculating the three main parameters: the degree orthokeratosis, the percentual drug activity and the mean epidermal thickness. The model is based on the property of certain substances to induce the differentiation of the granular layer which is absent or very poorly represented in psoriasis.

The morphometric evaluation was carried out by analyzing the images regarding:

- The length of the continuous granular layer developed between two hair follicles.

- The scale length defined as the distance between two hair follicles.

The following parameters were calculated from these measurements:

- **the degree of orthokeratosis**, which represents the degree of induction of normal epidermal differentiation, defined as a percentage ratio between the values obtained in the two previous measurements. Dimensions are calculated in micrometers.

- **the percentual drug activity** expressed as percentage values between the degrees of orthokeratosis expressed as values for the test groups, relative to the values of the negative. Through this parameter, the intensity of the anti-psoriatic activity can be compared in different experimental conditions.

- **the mean epidermal thickness** is calculated as the arithmetic mean of five measurements from the level of the lower part of the dermo-epidermal junction to the level of the stratum corneum, for one scale (between two hair follicles), thus achieving the evaluation of 10 scales for each mouse, 300 measurements in total. Mean epidermal thickness may be an indirect marker of granular layer growth.

The first experiment used as test substances diclofenac in concentrations of 1%, 2%, 4% and 8% respectively. The positive control group was treated with tretinoin 0.05%, one negative control group treated with the ointment base and another negative control group with untreated mice. The positive control group with tretinoin 0.05% has statistically significant results when compared to negative controls in terms of percentual orthokeratosis degree, mean epidermal thickness and drug activity, showing the validity of the test.

Results for diclofenac:

A. Orthokeratosis degree as a measure of the induction of epidermal differentiation was statistically significant compared to the negative control groups for the four concentrations of diclofenac tested ($p < 0.05$). The fact that diclofenac in concentrations of 1% and 2% had effects of stimulating the differentiation of the granular layer comparable to that of a topical retinoid created the premises to investigate the effects of higher concentrations of 4% and 8%, respectively, using the same experimental model. In conclusion, diclofenac has an anti-psoriatic action in terms of the induction of orthokeratosis, thus epidermal differentiation, dose-dependent with a minimum of action at 1% and a maximum at 8%, significant compared to the negative controls. In addition, the

concentrations of 4% and 8% were also statistically significant compared to tretinoin 0.05%, considered the basic standard on the action on the granular layer.

B. **The percentual drug activity** in inducing the granular layer development is around 27% - 28% for diclofenac 1% and 2% and 66% for diclofenac 4% and 8%. Diclofenac exhibits a concentration-dependent intensity of anti-psoriatic action, measured as granular layer induction, with superior results for diclofenac 4% and 8% when compared to the positive control, tretinoin 0.05% (with an intensity of action of approximately 36%).

C. **Mean epidermal thickness** for diclofenac 1%, 2%, 4% and 8% groups is statistically insignificant compared to the negative control groups. The 1%, 2%, and 8% groups were statistically significantly different from the 0.05% tretinoin group and nonsignificant from controls. Diclofenac 4% was not statistically significantly different from the positive control group in terms of mean epidermal thickness. The fact that these substances did not induce a statistically significant increase in the mean epidermal thickness compared to the negative controls cannot exclude a possible anti-psoriatic effect objectified by the increase of the granular layer.

The optimal concentration for the anti-psoriasis effect and the minimization of local or systemic adverse reactions, within the experimental model used, for diclofenac ointment is 4%, according to the data obtained. Following the research, we believe that the 4% concentration ointment is the optimal option because the orthokeratosis degree is insignificant compared to the 8% diclofenac ointment. This concentration is close to diclofenac formula approved by the FDA for the topical treatment of actinic keratoses.

Results for celecoxib.

A. The degree of orthokeratosis as a measure of the induction of the granular layer was significant for the celecoxib 2%, 4%, and 8% groups versus tretinoin 0.05%. The values obtained for the 4% and 8% concentrations are close and not statistically different. This fact shows a statistically significant anti-psoriasis effect for the three concentrations of 2%, 4% and 8%.

B. Percentual drug activity

Regarding the percentual drug activity, a dose-intensity relationship was observed for all concentrations of celecoxib, with celecoxib 8% having the most intense induction effect on the degree of orthokeratosis (70%) followed by celecoxib 4% (66.56 %).

C. Mean epidermal thickness

Regarding the mean epidermal thickness, statistical significance was obtained compared to untreated mice but not compared to those treated with white soft paraffin. The highest mean epidermal thickness values were for celecoxib 8%.

In conclusion, regarding the optimal concentrations of celecoxib that could later be tested in clinical trials as in the case of diclofenac, we opt for the concentration of 4% which offers an intensity of effect comparable to that of celecoxib 8% but with potentially few systemic side effects.

Results regarding the association of salicylic acid 2% with celecoxib 2%.

A. The degree of orthokeratosis was statistically significantly increased by 2% salicylic acid ointment, 2% celecoxib, and the combination of 2% celecoxib with 2% salicylic acid compared to the untreated mice group. Salicylic acid 2% increased the degree of orthokeratosis similar to tretinoin 0.05%. The addition of salicylic acid 2% to celecoxib 2% increased the degree of orthokeratosis statistically significantly more than the tretinoin 0.05% group.

B. The percentual drug activity was 52.16% for celecoxib 2% ointment, 32.97% for salicylic acid 2% ointment, and 85.14% for celecoxib 2% ointment combined with salicylic acid 2%. These results signify an additive relationship between the effect of 2% salicylic acid and 2% celecoxib.

C. Mean epidermal thickness was not significantly increased for any of the three groups (celecoxib 2%, salicylic acid 2%, and the combination of celecoxib 2% with salicylic acid 2%).

The combination of celecoxib 2% with salicylic acid 2% causes a summation effect. This effect is verified by the fact that when salicylic acid 2% or celecoxib 2% was tested separately, the degree of orthokeratosis was lower, while when combining the two substances the degree of orthokeratosis was much higher, increased by almost 20%.

Although the mechanism of obtaining the antiproliferative effect of the class of non-steroidal anti-inflammatory drugs is the inhibition of cyclo-oxygenase 2, it is possible that other mechanisms are also involved in this regard. Further research is needed to establish these mechanisms.

5.2. Personal contributions

We tested two substances from the class of nonsteroidal anti-inflammatory drugs (diclofenac and celecoxib) for their anti-psoriasis effect, an effect that had not been studied before, according to the databases available until the start of the study.

The research topic is represented by highlighting the anti-psoriasis effect of some substances from the NSAID class, namely diclofenac and celecoxib in mice. For this purpose, the tail model was used, a classic *in vivo* model based on morphometry, valid, which can allow the study of the *in vivo* effect of a substance on the epidermis in general as well as the granular layer in particular. It is thus possible to evaluate the induction of epidermal differentiation as a marker of the anti-psoriatic action as well as the intensity of action of a substance.

Diclofenac 3% is used in premalignant proliferations such as actinic keratoses. Celecoxib is currently in some non-clinical and clinical studies for topical use, but none of these studies have tested the efficacy of these substances *in vivo*, in psoriasis mice models.

The originality of this thesis is given by the fact that it is the first time that the two substances are tested in non-clinical studies *in vivo* in mice.

In this work, we developed a classic experimental model based on morphometry, but whose validity was tested by the statistically significant results obtained for the tretinoin positive control.

Based on this model, the evaluation was carried out using an objective measurement system through the working methodology used.

Resulting parameters and especially the degree of orthokeratosis was obtained by evaluating the presence of the granular layer as an indicator of anti-psoriatic activity. Another particularly important parameter was the percentual drug activity, expressed as a parameter derived from the degree of orthokeratosis, which allows evaluating the intensity in different experimental conditions and related to a maximal effect.

The results obtained strengthened the working hypotheses of the thesis, in the sense that anti-psoriatic effects were described for both diclofenac and celecoxib as follows:

- for diclofenac ointment, a percentual drug activity effect dependent on the concentration was obtained, the highest at 8%, superior even to tretinoin, which is the basic standard and the positive control.

- since the intensity of the anti-psoriasis effect was approximately equal for the concentrations of 4% and 8%, we can propose that for further non-clinical and clinical studies, diclofenac 4% should be considered, with optimal efficacy-safety ratio.

- for celecoxib ointment, an important dose-effect relationship was revealed and the percentual drug activity was relatively similar for concentrations of 2%, 4% and 8%, with the highest values for 8%. In the case of celecoxib, we propose the 4% concentration for future studies due to an optimal efficacy-safety ratio.

Celecoxib is a selective COX inhibitor recently approved for topical use, which underlines the importance of our research results on the topical anti-psoriasis effect and opens new research directions for new possible topical effects of this substance.

It should be noted that celecoxib belongs to selective inhibitors of cyclooxygenase 2, so it is possible that these topical effects to be class related, common for other coxibs as well, hypotheses that could be confirmed.

As an overview, it can be stated that the two non-steroidal anti-inflammatory drugs studied in this paper, diclofenac and celecoxib, substances already used in practice for several years and with very well demonstrated analgesic and anti-inflammatory effects, including topically, have new therapeutic values following this research, the results to be later tested in non-clinical but especially clinical studies.

In addition, celecoxib, a selective cyclooxygenase 2 inhibitor that had not been used until the start of the study in topical applications, demonstrated new therapeutic value in the topical treatment of psoriasis. This possible therapeutic use opens new horizons regarding the involvement of cyclooxygenase 2 in the pathogenesis of inflammatory skin diseases, including psoriasis, diseases that could benefit from other therapeutic approaches than the classical ones (glucocorticoids, calcineurin inhibitors, vitamin D analogs, etc.). The possible introduction into topical therapy of these cyclooxygenase inhibitors alone or in combination with salicylic acid but also with other substances already in use opens new perspectives in the therapy of psoriasis but also of other inflammatory conditions (dermatitis, drug eruptions).

The result when combining salicylic acid with celecoxib is interesting by obtaining an additive effect, salicylic acid 2% strengthens the effect of celecoxib 2% by adding anti-psoriatic actions. This association could allow, to decrease the concentrations of topically administered celecoxib with few possible systemic side effects after transcutaneous absorption, and would bring its own benefits, considering the percentual drug activity, by summing up the action on cyclooxygenase 2.

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1. **Nițescu, DAM**.; Mușetescu, A.; Nițescu, M.; Costescu, M.; Coman, OA. Experimental Research in Topical Psoriasis Therapy (Review). Exp. Ther. Med. 2021, 22, 971, PMID: 34335913, PMCID: PMC8290406, doi: 10.3892/etm.2021.10403, IF:2.7. (Study found in the first chapter).

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