MAEDICA – a Journal of Clinical Medicine
2022; 17(4): 805-811
https://doi.org/10.26574/maedica.2022.17.4.805

MAEDICA - a Journal of Clinical Medicine

ORIGINAL PAPER

The Effect of Topical Celecoxib as an Anti-Psoriasis Agent

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ABSTRACT

Our previous research showed that, in a mice tail model for psoriasis, topical celecoxib might have an anti-psoriatic effect in vivo *by increasing granular layer development. The aim of the present study is to assess the effect of three celecoxib concentrations using a mice model for psoriasis. Topical application of celecoxib (2%, 4% and 8%), with two negative controls (untreated and white soft paraffin as ointment base) and one positive control (tretinoin 0.05%), was performed for two weeks. The effect on granular layer development and epidermal thickness was measured on hematoxylin-eosin staining. The morphometric assessment was made by using mean orthokeratosis degree and mean epidermal thickness as main parameters. All celecoxib concentrations have significantly increased the mean orthokeratosis degree as a marker of an anti-psoriatic effect. Celecoxib 8% and 4% lead to a statistically significant increase in orthokeratosis degree when compared with celecoxib 2% and tretinoin 0.05%. Along with cyclo-oxygenase inhibition, other mechanisms of action of celecoxib are discussed. This study offers valuable information for future clinical trials with celecoxib as a topical anti-psoriasis agent.*

Keywords: celecoxib, psoriasis, experimental, topical.

INTRODUCTION

Topical therapy for moderate and localized forms of psoriasis is limited to a few classes of substances, whereas in severe forms it is used as an adjuvant to systemic therapy (1). The dermocorticoids are used as first-line topical treatment but they have serious local and systemic adverse effects, especially in long term use.

The tail mice model for psoriasis represents a valid *in vivo* model which particularly evaluates the effect of a certain substance on granular layer development and mean epidermal thickness, measured from the dermo-epidermal junction to the inferior part of the stratum corneum. The

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Article received on the 15th of October 2022 and accepted for publication on the 24th of November 2022

model also allows the evaluation of drug activity as a measure of the intensity of the anti-proliferative effect of a certain substance. The effect of a topical substance applied on mice tails is compared with control groups consisting of mice which were either untreated or treated only with the ointment base.

Celecoxib represents a non-steroidal anti-inflammatory substance that particularly inhibits cyclo-oxygenase 2, and is used in osteoarthritis and rheumatoid arthritis, reducing pain and inflammation (2, 3). Along with the cyclo-oxygenase inhibition celecoxib acts on other mechanisms like anti-apoptotic pathways in systemic or topical applications (4).

In our previous work regarding the anti-psoriasis effect of topical diclofenac or celecoxib, the results obtained in the case of celecoxib 2% showed an anti-psoriatic effect, by an increase in granular layer development (5). From this point, further data were needed in order to establish an optimal dose for the topical effect of celecoxib.

In our belief, the fact that some non-steroidal anti-inflammatory drugs have anti-proliferative effect when applied topically in premalignant entities like actinic keratosis is the premise for further investigation of the effect of these substances in diseases characterized by aberrant proliferation but also with inflammatory pathogeny such as psoriasis.

The aim of the present study was to assess the effect of multiple celecoxib concentrations using mice tail model for psoriasis. ❑

MATERIALS AND METHODS

Animals

Male Albino mice, weighing 20–30 grams, provided by Cantacuzino National Institute of Medico-Military Research and Development, Bucharest, Romania, were used for the present research. They were accommodated in individual cages four days before the experiment, with access to water and food in constant conditions (luminosity, temperature) for the entire duration of the experiment.

Substances

Substances of pharmacopeial quality, including white soft paraffin, celecoxib, and tretinoin, from a pure substance supplier (Fagron, Bucharest) were used. White soft paraffin was used as an ointment base for the incorporation of the other substances due to its advantage of creating an occlusion film that would assure a good absorption.

Experimental design

Animal groups

The experiment was developed for 14 days as described below.

Animals were divided into six groups, as follows:

- Group 1: negative control (untreated mice)
- Group 2: negative control (white soft paraffin)
- Group 3: positive control (tretinoin 0.05%)
- Group 4: celecoxib 2%
- Group 5: celecoxib 4%
- Group 6: celecoxib 8%

The untreated mice group and the soft white paraffin group were used as negative controls, for comparison with the normal histological aspect of the tail tissue. In groups 3-6, the tested substances were mixed within the white soft paraffin base. For the positive control group, tretinoin 0.05% in white soft paraffin, a known retinoid, was used.

Experimental protocol

The proximal part of the mice tails was treated with 0.1 milliliters of ointment each day with application of a plastic cylinder fixed with adhesive tape in contact with the treated surface and left for two hours, each day, five days *per* week for two weeks. After each daily application, the cylinders were removed and the treated region of the tail was washed with warm water. A weight evaluation of mice was made at two days, and a weight curve was similar for all groups. Mice were euthanized under general anesthesia, according to the ethical guidelines for lab animal research; then, tails were collected and fixed in 10% neutral-buffered formalin. The sections were cut at 4 μm and stained with Mayer hematoxylin and eosin after dehydration in graded ethanol, clarification in butanol and infiltration with paraffin.

Morphometric assessment

A Zeiss optical microscope was used for the evaluation of stained sections, and measurements were made in ZEN Blue software.

The following measurements in micrometers were done:

A) the horizontal length between two adjacent follicles is defined as scale for future measurements;
B) the horizontal length of the continuous granular layer present in the scale;
C) the vertical length of the epidermal thickness from the dermo-epidermal junction to the inferior part of the stratum corneum.

Secondary parameters were then obtained as follows:

D) The mean percentage degree of orthokeratosis is the ratio between the continuous horizontal granular layer length (B) and the scale length (A), in 10 horizontal scales *per* animal, 60 scales *per* group;
E) The percentage drug activity was measured as (OkS–OkC)/(100–OkC)×100

OkS=mean orthokeratosis values obtained for the tested substances in each group (S)
OkC=mean orthokeratosis values obtained for the negative control group 2 – white soft paraffin (C)

F) The mean epidermal thickness was calculated by five measurements *per* scale, 10 scales *per* animal, 300 measurements *per* group.

Statistical analysis

For the statistical analysis, Microsoft Excel, SPSS version 25, and the Kruskal–Wallis non-parametric test, with a level of significance set at $p < 0.05$, were used. ❑

RESULTS

Results regarding orthokeratosis degree and mean epidermal thickness were compared between the groups, as seen in Tables 1 and 2. The mean values of each group are shown in Table 3.

There was a significant difference in orthokeratosis degree between the negative control groups and those treated with celecoxib and tretinoin. Different concentrations of celecoxib lead to a rise in orthokeratosis degree in direct relation with the concentration of the topical agent. Orthokeratosis degree was the highest for celecoxib 8%, followed by celecoxib 4%, celecoxib 2%, tretinoin 0.05%, white soft paraffin and untreated group (Figure 1).

Drug activity was the highest for celecoxib 8%, followed by celecoxib 4%, celecoxib 2% and tretinoin 0.05% (Figure 2).

Group	Untreated	White soft paraffin	Celecoxib 2%	Celecoxib 4%	Celecoxib 8%	Tretinoin 0.05%
Untreated		N (0.055)	S (0.004)	S (0.004)	S (0.003)	S (0.004)
White soft paraffin	N (0.055)		S (0.004)	S (0.004)	S (0.003)	S (0.004)
Celecoxib 2%	S (0.004)	S (0.004)		S (0.004)	S (0.003)	S (0.025)
Celecoxib 4%	S (0.004)	S (0.004)	S (0.004)		N (0.116)	S (0.004)
Celecoxib 8%	S (0.003)	S (0.003)	S (0.003)	N (0.116)		S (0.003)
Tretinoin 0.05%	S (0.004)	S (0.004)	S (0.025)	S (0.004)	S (0.003)	

[1]S=statistically significant; N=statistically non-significant; level of significance is at p≤0.05.

TABLE 1. Multiple statistical comparisons regarding orthokeratosis using Kruskal-Wallis test

Group	Untreated	White soft paraffin	Celecoxib 2%	Celecoxib 4%	Celecoxib 8%	Tretinoin 0.05%
Untreated		N (0.109)	N (0.15)	S (0.016)	S (0.003)	S (0.004)
White soft paraffin	N (0.109)		N (0.2)	N (0.873)	N (0.317)	N (0.631)
Celecoxib 2%	N (0.15)	N (0.2)		N (0.109)	S (0.032)	S (0.013)
Celecoxib 4%	S (0.016)	N (0.873)	N (0.109)		N (0.475)	N (0.423)
Celecoxib 8%	S (0.003)	N (0.317)	S (0.032)	N (0.475)		N (0.886)
Tretinoin 0.05%	S (0.004)	N (0.631)	S (0.013)	N (0.423)	N (0.886)	

[1]S=statistically significant; N=statistically non-significant; level of significance is at p≤0.05.

TABLE 2. Multiple statistical comparisons regarding mean epidermal thickness using Kruskal-Wallis test

Group	Percentage orthokeratosis	Mean epidermal thickness	Percentage drug activity
Untreated	13.27±1.4	26.33±2.34	
White soft paraffin	15.19±1.04	31.2±4.7	0
Celecoxib 2%	59.43±4.02	28.23±1.97	52.16
Celecoxib 4%	71.64±3.43	31.66±3.58	66.56
Celecoxib 8%	75.06±3.37	33.43±3.69	70.59
Tretinoin 0.05%	48.72±6.09	33.22±2.61	39.54

TABLE 3. Mean values regarding percentage orthokeratosis, mean epidermal thickness and percentage drug activity

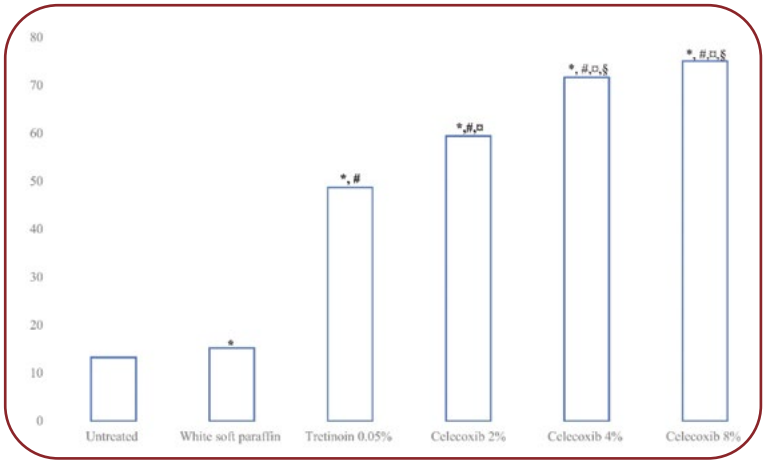

FIGURE 1. Percentage orthokeratosis degree in eight groups. Statistically significant ($p < 0.05$) when compared to: *untreated group; #white soft paraffin group; ¤tretinoin 0.05% group; §celecoxib 2% group

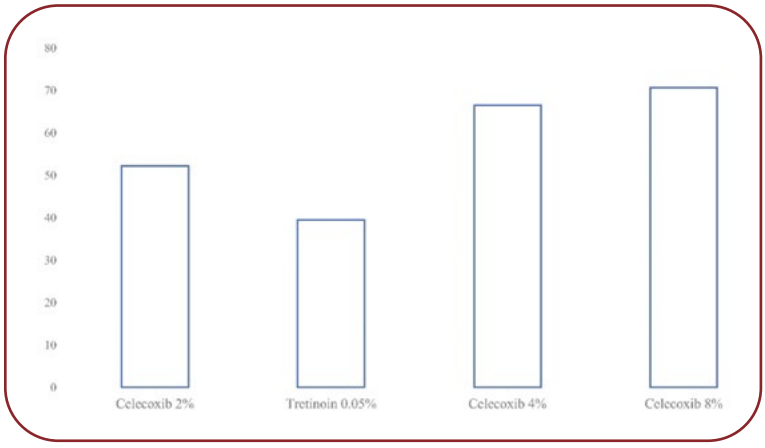

FIGURE 2. Percentage drug activity of celecoxib 2%, 4%, and 8% using the following formula: (OkS–OkC)/(100–OkC)×100. OkS=mean orthokeratosis values obtained for the tested substances in each group (S); OkC=mean orthokeratosis values obtained for the negative control group 2 – white soft paraffin (C)

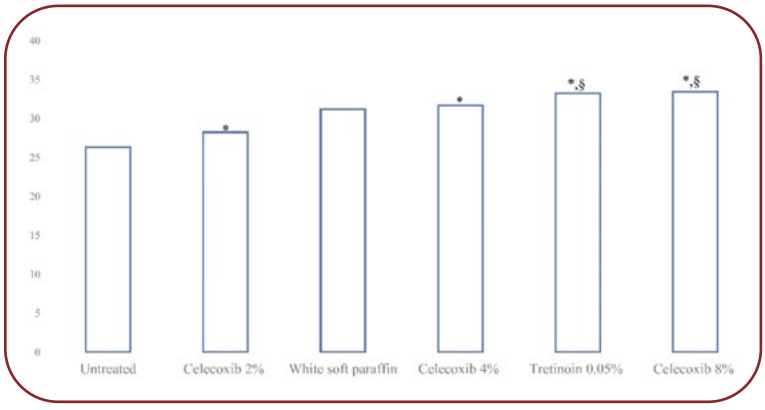

FIGURE 3. Mean epidermal thickness for six groups. Statistically significant ($p < 0.05$) when compared to: *untreated group; §celecoxib 2% group.

The mean epidermal thickness decreased in the following order: celecoxib 8%, tretinoin 0.05%, celecoxib 4%, white soft paraffin, and celecoxib 2%. Except for celecoxib 2% and white soft paraffin, the remaining groups have shown a significantly increased mean epidermal thickness when compared to untreated mice. In addition, celecoxib 8% significantly increased the mean epidermal thickness when compared to celecoxib 2% (Figure 3). ❑

DISCUSSION

Psoriasis represents a very challenging pathology regarding the treatment. In localized and limited forms of the disease, choosing a good topical treatment with minimal adverse effects, is the most wanted approach. Preclinical studies regarding a potential anti-psoriatic effect of a certain substance can be made *in vivo* using mice models that can assess some aspects of psoriatic disease pathogeny (6). In our study, we used the mouse tail model for psoriasis based on analysis of substance effect on granular layer development using orthokeratosis degree as a parameter to object an anti-psoriatic effect.

In our previous studies, we found that celecoxib 2% had a comparable anti-psoriatic effect with tretinoin 0.05%, a documented anti-psoriasis substance, used as a positive control.

In the present study, we aimed to establish the optimal concentration of celecoxib that could have anti-psoriatic potential.

Orthokeratosis degree was highest for celecoxib 8%, followed by celecoxib 4% and celecoxib 2%. The values obtained for celecoxib 8% and celecoxib 4% are very close to each other and there are no significant differences between them.

Mean epidermal thickness, a parameter measured from the dermo-epidermal junction to the inferior part of the stratum corneum, was corroborated with the orthokeratosis degree in order to evaluate the anti-psoriatic effect of a certain substance. All tested substances, except for white soft paraffin, significantly increased the mean epidermal thickness as compared to untreated mice.

Mean epidermal thickness was the highest for celecoxib 8%, followed by tretinoin 0.05%. Celecoxib 4% lead to an increase in both the mean epidermal thickness and orthokeratosis degree. Increases in mean epidermal thickness were significant only in comparison with the untreated mice. The use of white soft paraffin resulted in a slight non-significant increase of the mean epidermal thickness, but not also of the orthokeratosis degree; therefore, it cannot be considered to have an anti-psoriatic effect.

Given that celecoxib 4% and 8% ointment induced orthokeratosis, they might have anti-psoriatic effects, with statistically significant results when compared to celecoxib 2% alone. These results suggest that both celecoxib 8% and 4% could be used with similar results. Moreover, celecoxib 4% has a smaller concentration of active substance than colecoxib 8%, while producing similar results and fewer adverse reactions, which makes it a preferred topical treatment choice.

Percentage drug activity, a secondary parameter derived from the orthokeratosis degree, shows the intensity of orthokeratosis effect of a given substance in relation to the possible maximal effect. Celecoxib 8% had a more intense effect than the other tested substances, closely followed by celecoxib 4%. All celecoxib concentrations had percentage drug activities higher than 50%, with a maximum for celecoxib 8% (70.59%) and a minimum for celecoxib 2% (52.16%). This parameter allows a good comparison between the effect of a certain substance in different experimental settings.

Inactivation of AMPK aggravates psoriasis by inhibiting autophagy (7). Autophagy associated proteins like ULK1/Atg7 and PINK1/Parkin are activated by AMPK phosphorylation with inhibition of inflammatory factors and alleviation of clinical signs of psoriasis (7).

Cyclo-oxygenase 2 inhibitors such as celecoxib can interfere with AMPK activation. This mechanism can explain the potentiation of anti-psoriatic action observed in the experiment. This fact brings new data that justify the utility of further research regarding substances that can influence this pathway and have anti-psoriatic potential.

Celecoxib was tested in some studies for the anti-neoplastic effect in skin UVA-UVB carcinogenesis with significant inhibition of skin carcinogenesis in oral administration (8–12) but also in locally applications (13, 14). Topically applied celecoxib was used in order to limit its systemic toxicity, also for neoplastic pathologies like oral cancer (15).

Overexpression of COX-2 can increase prostaglandin E2 (PGE-2) synthesis and could be involved early in tumorigenesis and in hyperproliferation, cell migration and invasion. PGE-2 activates PI3 (phosphatidylinositol 3-kinase)/Akt pathway through the EGFR (epidermal growth factor receptor) (16).

These findings suggest that overexpression of COX-2 enzyme plays an important role in tumor progression by multiple pathways, including (EGFR)-proto-oncogene RAS-extracellular signal regulated kinase (ERK) (EGFR-Ras-ERK), phosphoinositide 3-kinase-protein kinase (PK) B (PI3-K-Akt), cAMP-PKA, transcription factors, activating protein-1 (AP-1) and nuclear factor kB (NF-kB) (17). AP-1 transcription factor, a key determinant in psoriasis pathogeny, regulates COX-2 transcription in regards not only for epithelial cells but also dendritic cells (18, 19). ❑

CONCLUSIONS

Celecoxib, as a topical agent, increases the orthokeratosis degree and therefore, it may have an anti-psoriatic effect *in vivo*. Celecoxib 8% and 4% have similar results as possible anti-psoriatic agents and better results when compared with celecoxib 2% alone.

The mechanism involved in the anti-psoriatic effect of celecoxib may be dependent on cyclooxygenase 2 inhibition, but also on other mechanisms involved in cell differentiation and autophagy.

The present study offers valuable information for future clinical trials of celecoxib as a topical anti-psoriatic agent. ❑

Conflicts of interest: none declared.

Financial support: none declared.

Authors' contributions: D.A.-M.N. made the experimental design, conducted the experimental protocol, analyzed the obtained specimens and data. H.P. made the experimental design and conducted the statistical and data analysis. D.G. made the histopathological sections and staining. A.M contributed to the histopathological sections analysis. A.C.S. helped with the experimental protocol and animal housing. O.A.C. made the experimental design, supervised the experiment, and analyzed the results. All authors drafted the work or revised it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania (13310/27 May 2021) for studies involving animals, according to 43/2014 Law regarding animal protection used in scientific purposes, with further completions and 86/609/CEE Directive from 24 November 1986 regarding acts with power of law and administrative acts of member states for animal protection used in experimental purposes and other scientific purposes.

Data availability: Data will be available upon request.

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