

XemaTop™

Evaluation of Different Formulations Applied to Psoriasis Tissue (Part 2/3)

Abstract: Psoriasis is a chronic immune-mediated inflammatory disease of the skin characterized by hyperproliferation of keratinocytes. The inhibition of the protein Ki67, a biomarker of cell proliferation, is associated with antiproliferative properties. A total of 4 topical formulations were applied to an *in vitro* psoriasis tissue model, followed by detection of Ki67 by an immunosorbent assay. All formulations significantly inhibited the production of Ki67, with the XemaTop compounded formulations outperforming the commercial medicines of reference. The proprietary base is therefore likely to facilitate the delivery of active substances to psoriatic skin and may then be recommended in the management of psoriasis.

Introduction:

Psoriasis is a chronic immune-mediated inflammatory disease of the skin characterized by red, scaly, and well-defined lesions that form as a result of epidermal hyperproliferation. Keratinocytes, cells within the epidermal layer of the skin, may be stained for the expression of growth regulating proteins such as Ki67. This protein is up-regulated in psoriatic skin and is therefore considered a biomarker of cell proliferation [1]. Topical corticosteroids (e.g. mometasone furoate) and vitamin D analogues (e.g. calcitriol) are commonly prescribed in the management of psoriasis due to their antiproliferative properties [2].

The purpose of this study is to evaluate the *in vitro* antiproliferative properties of different formulations applied to psoriasis tissue using reconstructed psoriasis tissue model, a 3-dimensional (3D) model obtained from human skin tissue specimens with the following characteristics associated with psoriasis: increased cellular proliferation and cytokine release, and presence of psoriasis-associated biomarkers [3]. A total of 4 formulations were tested, including 2 commercial medicines and 2 compounded medicines, as follows: Mometasone Furoate Ointment USP 0.1% (Perrigo®); Vectical® Ointment Calcitriol 3 mcg/g; Mometasone Furoate 0.1% in XemaTop; and Calcitriol 3 mcg/g in XemaTop. The commercial medicines selected are commonly prescribed in psoriasis and were used in this study as positive controls. XemaTop is a proprietary base developed to be used in compounded topical formulations for patients with common skin disorders, such as psoriasis.

Methodology:

An aliquot of 50 µL of each test formulation (4 replicates) was applied to reconstructed psoriasis tissue samples (MatTek Corporation), on day 0 and on day 2 of the study. Four additional tissue samples were left untreated to serve as study control. Psoriasis tissues were collected on day 5 for detection of Ki67 using Immunohistochemical Analysis [4] and the Enzyme-Linked Immunosorbent Assay (ELISA) [5].

Immunohistochemical Analysis: rabbit mAb (IHC Specific) recognizes endogenous levels of total Ki-67 protein and was used in accordance to protocol ID 283 [4]. Proliferating cells were stained brown and digital images were taken at 10x magnification.

ELISA: using a 96-well plate, the bottom of each well was coated with a rat monoclonal antibody that binds to any Ki67 introduced into the well. The proteins isolated from psoriasis tissues were applied to the antibody coated plates, followed by incubation and washing. A second, non-overlapping biotin-conjugated rat monoclonal antibody was then added to the wells followed by horseradish peroxidase (HRP)-conjugated streptavidin and the chromogenic substrate TMB (3, 3', 5, 5' - tetramethylbenzidine), which generated a reaction that resulted in a yellow color once terminated with acid. The intensity of the yellow color was measured with a plate reader at 450 nm [5,6].

Results and Discussion:

The levels of Ki67 produced by the psoriasis tissue samples following application of the 4 test formulations were quantified based on the absorbance detected at 450 nm. The intensity of the yellow color generated by the ELISA is directly proportional to the absorbance level, which is proportional to the concentrations of Ki67 in the collected samples [7]. Mean Ki67 concentrations (%) ± SD were calculated for each test formulation and compared to the untreated tissue samples, as displayed in Table 1 and Figure 1.

According to the results obtained, concentrations of Ki67 in the psoriasis tissue samples treated with the 4 test formulations were considerably lower than the concentrations in the untreated tissues, which shows that all formulations inhibited the production of Ki67. Taking into account that the growth regulating protein Ki67 is indicative of proliferating cells, a reduction of Ki67 in the psoriasis tissue samples suggests that all test formulations presented antiproliferative properties. A reduction of Ki67 was expected in the psoriasis tissue samples treated with the positive controls (Mometasone Furoate Ointment USP 0.1% and Vectical® Ointment Calcitriol 3 mcg/g) as both correspond to commercial medicines with antiproliferative properties, commonly prescribed in psoriasis [2]. When Mometasone Furoate 0.1% in XemaTop and Calcitriol 3 mcg/g in XemaTop are compared to the corresponding commercial medicines (Mometasone Furoate Ointment USP 0.1% and Vectical® Ointment Calcitriol 3 mcg/g, respectively), it is demonstrated that the compounded medicines inhibited the production of Ki67 to a greater extent (48.04% vs 74.07%; 32.42% vs 78.27%).

The ELISA results are in accordance with the immunohistochemical analysis, which show that the highest inhibition of proliferating cells was obtained for the XemaTop formulations (Figure 2).

Conclusions:

The *in vitro* psoriasis tissue model is a valuable tool to evaluate the effect of topical formulations in psoriasis. The negative control (untreated tissues) and the positive controls (commercial medicines of reference) used in this study confirmed that the tissue samples were metabolically active and responsive to therapeutic agents. The highest inhibition of the biomarker Ki67 was achieved with the XemaTop formulations, suggesting that the proprietary base facilitates the delivery of active substances to psoriatic skin and hence the better performance of the base when compared to the commercial medicines of reference. The inhibition of Ki67 is likely to attenuate the cellular proliferation associated with psoriasis and, as a result, XemaTop may be considered a valuable proprietary base for the incorporation of active substances when compounding topical formulations indicated in psoriasis.

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Test Formulations	Relative Ki67 concentration ± SD
Negative control (untreated tissues)	100.01 % ± 33.85
Mometasone furoate 0.1% in XemaTop	48.04 % ± 3.01
Calcitriol 3 mcg/g in XemaTop	32.42 % ± 1.11
Mometasone Furoate Ointment USP 0.1%	74.07 % ± 10.57
Vectical® (calcitriol) Ointment 3 mcg/g	78.27 % ± 5.56

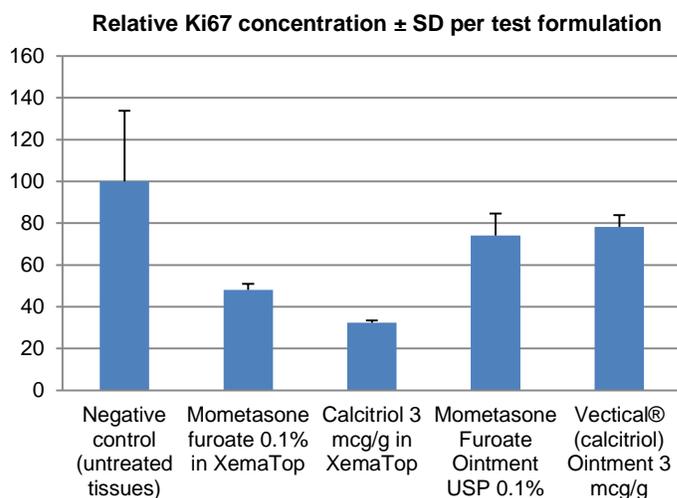


Table 1. (left) and **Figure 1.** (right) Relative Ki67 concentrations ± SD detected by the ELISA assay following application of the test formulat

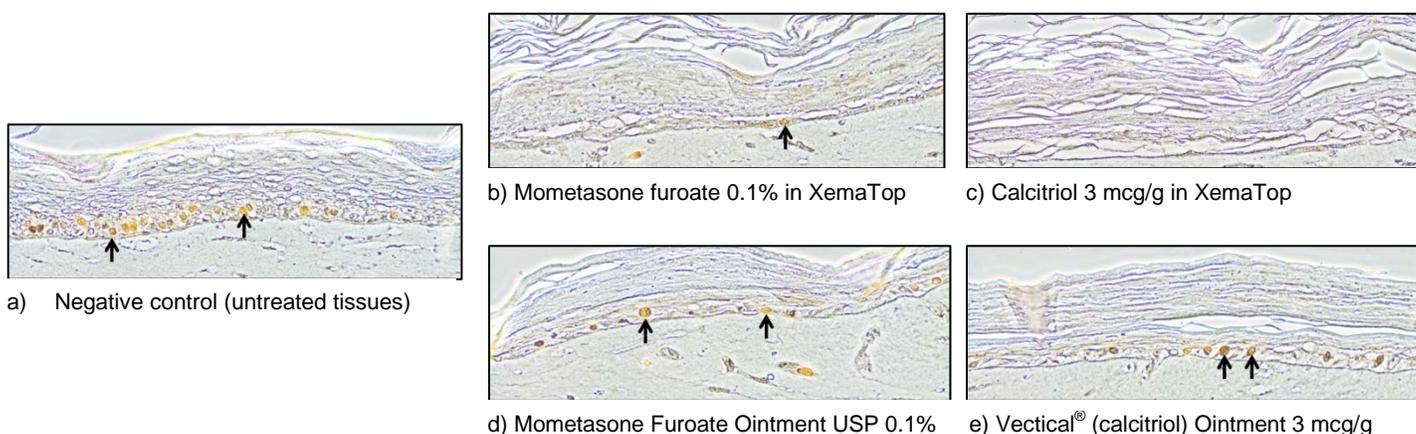


Figure 2. Immunohistochemical analysis of the psoriasis tissue following application of the test formulations (a-e); proliferating cells were stained brown with rabbit mAb (IHC Specific) and digital images were taken at 10x magnification.

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