

XemaTop™

Evaluation of Different Formulations Applied to Psoriasis Tissue (Part 1/3)

Abstract: Psoriasis is a chronic immune-mediated inflammatory disease of the skin characterized by hyperproliferation of keratinocytes. The inhibition of cytokines such as the interleukin (IL)-6 is associated with anti-inflammatory and antiproliferative properties. A total of 4 topical formulations were applied to an *in vitro* psoriasis tissue model, followed by detection of IL-6 by an immunosorbent assay. All formulations significantly inhibited the production of IL-6, 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 hyperproliferation of keratinocytes (cells within the epidermal layer of the skin). Though the exact mechanism is poorly understood, it has been proposed that cytokines such as interleukin (IL)-2, IL-6, IL-8, and tissue necrosis factor (TNF)- α play a vital role in facilitating the inflammatory response and the hyperproliferation of keratinocytes [1]. Topical corticosteroids such as mometasone furoate, triamcinolone acetonide, and clobetasol propionate are often used in the management of psoriasis due to their anti-inflammatory and antiproliferative properties. Vitamin D analogues such as calcitriol can also be applied topically for psoriasis treatment due to their antiproliferative properties [2].

The purpose of this study is to evaluate the *in vitro* anti-inflammatory and antiproliferative properties of different formulations applied to psoriasis tissue using reconstructed psoriasis tissue model and Enzyme-Linked Immunosorbent Assay (ELISA) for detecting IL-6 production. The psoriasis tissue model (Figure 1) is 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.

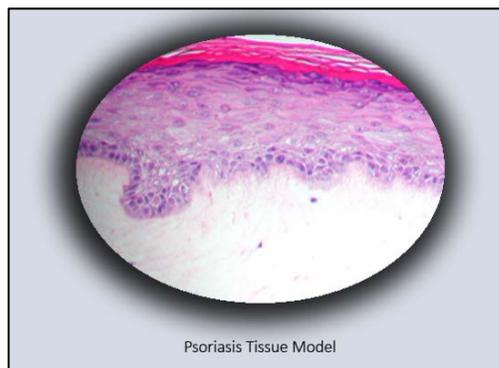


Figure 1. Illustration of the psoriasis tissue model.

Methodology:

An aliquot of 50 μ L 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. Culture media were collected on day 5 for IL-6 detection using the ELISA assay by Cayman Chemical [4].

ELISA assay for detecting IL-6 production

The ELISA assay operates based on a double-antibody technique [5]. Using a 96-well plate, the bottom of each well was coated with a rat monoclonal antibody that binds to any IL-6 introduced into the well. The collected culture media 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 [4]. The intensity of the yellow color was measured with a plate reader at 450 nm.

Results and Discussion:

The levels of IL-6 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 assay is directly proportional to the absorbance level, which is proportional to the concentrations of IL-6 in the collected samples [6]. Mean IL-6 concentrations (pg/mL) \pm SD were calculated for each test formulation and compared to the untreated tissue samples, as displayed in Table 1 and Figure 2. Statistical significance was determined using *p*-values obtained from a student's *t*-Test. A *p*-value of less than 0.05 ($p < 0.05$) is considered statistically significant.

According to the results obtained, concentrations of IL-6 in the psoriasis tissue samples treated with the 4 test formulations were significantly lower than the concentrations in the untreated tissues, with $p < 0.05$ (statistically significant), which shows that all formulations inhibited the production of IL-6. Considering that cytokines facilitate the inflammatory response and the hyperproliferation of keratinocytes, a reduction of IL-6 in the psoriasis tissue samples suggests that all test formulations presented anti-inflammatory and antiproliferative properties.

A reduction of IL-6 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 anti-inflammatory and antiproliferative properties, commonly prescribed in psoriasis [2].

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Table 1. Mean IL-6 concentrations ± SD detected following application of the test formulations.

Test Formulations	Mean IL-6 (pg/mL) ± SD	P-value (negative control)	P-value (positive controls)
Negative control (untreated tissues)	528.864 ± 12.153	—	—
Mometasone furoate 0.1% in XemaTop	79.713 ± 9.596	1.763E ⁻⁰⁹	0.040
Calcitriol 3 mcg/g in XemaTop	54.023 ± 7.096	7.125E ⁻¹⁰	0.001
Mometasone Furoate Ointment USP 0.1%	113.902 ± 15.439	5.200E ⁻⁰⁶	—
Vectical® (calcitriol) Ointment 3 mcg/g	106.898 ± 15.212	1.009E ⁻⁰⁸	—

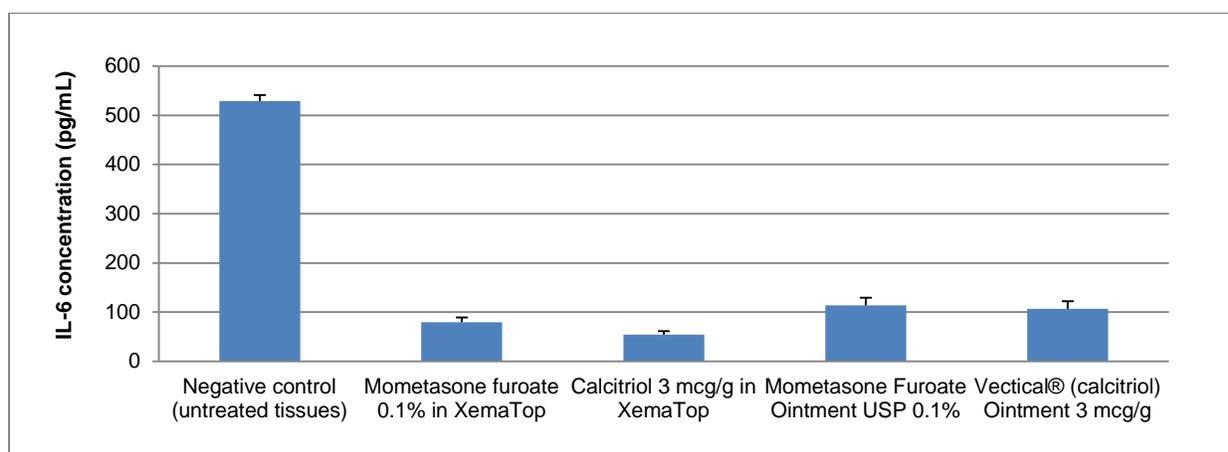


Figure 2. Mean concentration of IL-6 ± SD detected in collected culture media following the application of 4 test formulations.

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 IL-6 to a greater extent (79.713 pg/mL vs 113.902 pg/mL, p=0.040; 54.023 pg/mL vs 106.898 pg/mL, p=0.001). These results suggest that the XemaTop formulations outperformed the anti-inflammatory and antiproliferative properties of the reference products.

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 IL-6 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 IL-6 is likely to attenuate the inflammatory response and 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.

References:

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