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79th FIP World Congress of Pharmacy and Pharmaceutical Sciences

Abu Dhabi, United Arab Emirates
22 - 26 September 2019
PHARMACEUTICAL COMPOUNDING: AN ALTERNATIVE TREATMENT OPTION IN PSORIASIS

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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 hyper-proliferation of keratinocytes.

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 hyper-proliferation of keratinocytes.

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 anti-proliferative properties.

Vitamin D analogues such as calcitriol can also be applied topically for psoriasis treatment due to their anti-proliferative properties.
OBJECTIVE

• To evaluate the *in vitro* anti-inflammatory and anti-proliferative properties of different formulations applied to psoriasis tissue using reconstructed psoriasis tissue model and Enzyme-Linked Immunosorbent Assay (ELISA) for detecting IL-6 production.
Psoriasis Tissue Model

- 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.
A total of 4 formulations were tested, including 2 commercial products and 2 compounded dosage forms, 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 products selected are commonly prescribed in psoriasis and were used in this study as positive controls.

XemaTop is a proprietary base developed by PCCA to be used in compounded topical formulations for patients with common skin disorders, such as psoriasis.
METHODS & MATERIALS

• **Base, PCCA XemaTop™ Ingredients**

  - Water, Cyclopentasiloxane, Glyceryl Stearate, PEG-100 Stearate, Polyglyceryl-3 Cetearyl Ether Olivate, Propanediol, Carapa Guianensis Seed Oil, Dimethicone, Hexyldecanol, Lactobacillus Ferment, Polyacrylate Crosspolymer-11, Butylene Glycol, Pentylene Glycol, Glycerin, Theanine, Cholecalciferol, Beta-Glucan, Phosphatidylcholine, Phosphatidyglycerol, Brassica Campestris (Rapeseed) Sterols, Vitis Vinifera (Grape) Seed Extract, Boswellia Serrata Resin Extract, Bisabolol, Xylitol, Anhydroxylitol, Xylitylglucoside, Tocopheryl Acetate, Cetylhydroxyproline Palmitamide, Hydroxyphenyl Propamidobenzoic Acid, Maltodextrin, Stearic Acid, Polysilicone-11, Microcrystalline Cellulose, Silica, Disodium EDTA, Sodium Benzoate

  - This exclusive PCCA base replenishes the skin’s lipids and structural integrity, while improving the appearance of red, irritated skin.
• 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.  
• Culture media were collected on day 5 for IL-6 detection using the ELISA assay by Cayman Chemical.
METHODS & MATERIALS

- **ELISA assay for detecting IL-6 production**
- The ELISA assay operates based on a double-antibody technique.
- 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.
- The intensity of the yellow color was measured with a plate reader at 450 nm.
• 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.
RESULTS AND DISCUSSION

• Mean IL-6 concentrations (pg/mL) ± 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.
RESULTS AND DISCUSSION

• 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 hyper-proliferation of keratinocytes, a reduction of IL-6 in the psoriasis tissue samples suggests that all test formulations presented anti-inflammatory and anti-proliferative properties.
RESULTS AND DISCUSSION

• 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 products with anti-inflammatory and anti-proliferative properties, commonly prescribed in psoriasis.

• The two compounded dosage forms Mometasone Furoate 0.1% in XemaTop and Calcitriol 3 mcg/g in XemaTop 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) than the commercial products.
RESULTS AND DISCUSSION

• A second study was conducted with the same 4 formulations using a different biomarker, namely the growth regulation protein Ki67.

• This protein is up-regulated in psoriatic skin and is therefore considered a biomarker of cell proliferation.

• The results are summarized in Table 1 and Figures 1 and 2.

Table 1. (left) and Figure 1. (right) Relative Ki67 concentrations ± SD detected by ELISA assay following application of the test formulations.
RESULTS AND DISCUSSION

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.
CONCLUSION

- The *in vitro* psoriasis tissue model is a valuable tool to evaluate the effect of topical formulations in psoriasis.
- Maximum inhibition of the cytokine IL-6 and the biomarker Ki67 was achieved with the XemaTop formulations.
- This inhibition attenuates the inflammatory response and cellular proliferation associated with psoriasis.
- The proprietary PCCA base XemaTop facilitates the delivery of active substances to psoriatic skin and out-performs the anti-inflammatory and anti-proliferative properties of the commercial reference products.