TECHNICAL REPORT

Evaluation of Naltrexone HCl 1% Topical Cream (#11934) Applied to Psoriasis Tissue In Vitro

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 and the growth regulating protein Ki67 is associated with anti-inflammatory and antiproliferative properties. Naltrexone HCl 1% Topical Cream (XemaTop™) (PCCA Formula 11934) reduced the concentration of IL-6 and Ki67 in the psoriasis tissue (versus untreated tissues) by 70.3% and 68.6%, respectively. The results of this study show that the formulation inhibited the expression of both markers in vitro and is therefore likely to attenuate the inflammatory response and cellular proliferation associated with psoriasis in vivo.

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) (Fig.1). Keratinocytes may be stained for the expression of growth regulating proteins such as Ki67 and are therefore considered a biomarker of cell proliferation. Though the exact mechanism of psoriasis is poorly understood, it has been proposed that cytokines such as interleukin (IL)-6 play a vital role in facilitating the inflammatory response and the hyperproliferation of keratinocytes1,2.

Naltrexone is a long-acting, non-addictive oral opioid receptor antagonist commonly administered at a recommended dose of 50 mg daily by mouth3. Low dose naltrexone (LDN) refers to daily dosages of naltrexone that are approximately 1/10th of the recommended (≈4.5 mg), which have demonstrated ‘paradoxical’ properties, including analgesia and anti-inflammatory effects4. LDN has been successfully used in autoimmune diseases such as rheumatoid arthritis and psoriasis5.

The purpose of this study is to evaluate the in vitro anti-inflammatory and antiproliferative properties of Naltrexone HCl 1% Topical Cream (XemaTop™) (PCCA Formula 11934) applied to psoriasis tissue using reconstructed psoriasis tissue model, a 3-dimensional (3D) model obtained from human skin tissue specimens with the following characteristics: increased cellular proliferation and cytokine release, and presence of psoriasis-associated biomarkers6.

Methodology:
An aliquot of 50 µL of PCCA Formula 11934 (2 replicates) was applied to reconstructed psoriasis tissue samples (SOR-300-FT, MatTek Corporation), on days 0 and 2. Two additional tissue samples were left untreated to serve as study control. Culture media were collected on day 5 for IL-6 detection. Further reconstructed psoriasis tissue samples were harvested on day 2 for Ki67 expression testing using the Immunohistochemical Analysis. The Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect both the IL-6 (Abcam) and the ki67 (LifeSpan BioSciences, Inc.).

Immunohistochemical Analysis: rabbit mAb (IHC Specific) recognizes endogenous levels of total Ki-67 protein and was used in accordance to protocol ID 283. Proliferating cells were stained brown and digital images were taken at 10x magnification7.

ELISA: this 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 and Ki67 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 color was measured with a plate reader at 450 nm8-10.

Results and Discussion:
The concentration of IL-6 and Ki67, proportional to the intensity of the yellow color generated by the ELISA assay, was calculated for the test formulation and compared to the untreated tissue samples, as displayed in Table 1. Statistical significance was determined using p-values obtained from a student’s t-Test.

The mean concentration of IL-6 and Ki67 in the tissue samples treated with PCCA Formula 11934 was significantly lower in comparison to the untreated tissues, with \( p < 0.05 \) (statistically significant). According to Table 1, the Naltrexone HCl 1% Topical Cream (XemaTop™) reduced the levels of IL-6 and Ki67 by 70.3% and 68.6%, respectively, which shows that the formulation inhibited the expression of both markers in vitro.

Figure 1. Schematic representation of psoriasis (adapted from BlueRingMedia/Shutterstock.com)
Table 1. Mean concentration of IL-6 and Ki67 detected following application of PCCA Formula 11934 versus untreated tissues

<table>
<thead>
<tr>
<th>Test Formulations</th>
<th>Mean IL-6 (pg/mL) ± SD</th>
<th>Mean Ki67 (ng/mL) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (untreated tissues)</td>
<td>166.33 ± 12.19</td>
<td>2.12 ± 0.18</td>
</tr>
<tr>
<td>Naltrexone HCl 1% Topical Cream</td>
<td>48.59 ± 9.84</td>
<td>0.82 ± 0.18</td>
</tr>
<tr>
<td>(XemaTop™)</td>
<td>6.35E-06</td>
<td>0.02</td>
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<tr>
<td>P-value</td>
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</tbody>
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Considering that cytokines facilitate the inflammatory response and that the growth regulating protein is indicative of proliferating cells, a reduction of IL-6 and Ki67 in the psoriasis tissue samples suggests that the PCCA Formula 11934 presented anti-inflammatory and antiproliferative properties. Naltrexone HCl 1% in XemaTop™, a proprietary base developed to be used in compounded topical formulations for patients with common skin disorders, may then be recommended in psoriasis.

Conclusions:

The *in vitro* psoriasis tissue model is a valuable tool to evaluate the effect of topical formulations in psoriasis. The inhibition of the markers IL-6 and Ki67 following *in vitro* application of PCCA Formula 11934 suggests that Naltrexone HCl 1% in XemaTop is likely to attenuate the inflammatory response and cellular proliferation associated with psoriasis in *vivo*. In previous studies, it was demonstrated that XemaTop facilitated the delivery of active substances to psoriatic skin (PCCA Technical Reports 99069, 99156 and 99157). As a result, compounding pharmacists have additional evidence to support the use of XemaTop for the incorporation of active substances when compounding topical formulations indicated in psoriasis.

References: