TECHNICAL REPORT

XemaTop™

Evaluation of Pentoxifylline 10% Topical Cream (#12374) Applied to Psoriasis Tissue *In Vitro*

Abstract: Psoriasis is a chronic immune-mediated inflammatory disease of the skin characterized by scaly lesions that form as a result of hyperproliferation of keratinocytes, increased collagen and fibronectin. Pentoxifylline 10% Topical Cream (XemaTop™) (PCCA Formula 12374) reduced the concentration of inflammatory cytokines (IL-1β, IL-6 and GM-CSF), pro-collagen type I and fibronectin on a 3D reconstructed psoriasis tissue model, in comparison to untreated tissue samples. Immunohistochemical analysis also suggested a reduction in collagen and fibronectin as demonstrated by the thinner epidermis in the treated tissues. Pentoxifylline 10% in XemaTop 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 scaly lesions that form as a result of hyperproliferation of keratinocytes (cells within the epidermal layer of the skin), increased collagen and fibronectin (Figure 1). Keratinocytes may be stained for the expression of inflammatory cytokines such as the interleukin (IL)-1β, IL-6 and the granulocyte/macrophage colony-stimulating factor (GM-CSF)[1]. The purpose of this study is to evaluate the *in vitro* antipsoriatic properties of Pentoxifylline 10% Topical Cream (XemaTop™) (PCCA Formula 12374) using a 3-dimensional (3D) reconstructed psoriasis tissue model. Pentoxifylline is a methylxanthine derivative with a variety of anti-inflammatory and antifibrinolytic effects[2], whereas XemaTop™ is a proprietary base developed to be used in compounded topical formulations for patients with common skin disorders, such as psoriasis.

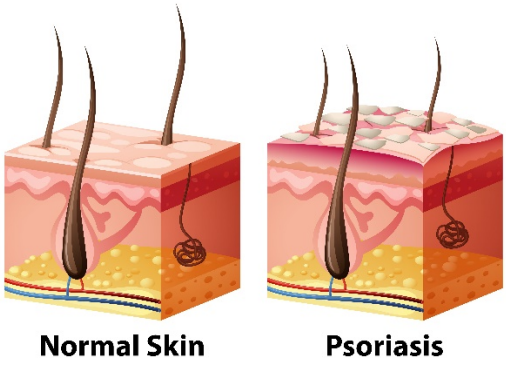

Figure 1. *Schematic representation of normal skin versus psoriasis (adapted from BlueRingMedia/Shutterstock.com)*

Methodology:

An aliquot of 50 µL of PCCA Formula 12374 (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 days 2 and 5 for detection of pro-collagen type I, fibronectin, and the inflammatory cytokines (IL1β, IL-6 and GM-CSF) by the Enzyme-Linked Immunosorbent Assay (ELISA, Abcam). Further reconstructed psoriasis tissue samples were harvested on day 2 for collagen and fibronectin testing using the Immunohistochemical Analysis.

Results and Discussion:

The concentration of the inflammatory cytokines, collagen and fibronectin was calculated for the test formulation and compared to the untreated tissue samples, as displayed in Tables 1 and 2. Statistical significance was determined using *p*-values obtained from a student's t-Test.

The mean concentration of IL-1β, IL-6 and GM-CSF in the tissue samples treated with PCCA Formula 11934 was significantly lower in comparison to the untreated tissues, with $p < 0.05$ (statistically significant), on both days 2 and 5 (with exception of IL-1β). According to Table 1, the Pentoxifylline 10% Topical Cream (XemaTop™) reduced the concentration of IL-1β, IL-6 and GM-CSF on day 5 by 20.1%, 94.5% and 95.9%, respectively, which shows that the formulation inhibited the expression of all biomarkers *in vitro*. Likewise, the mean concentration of pro-collagen type I and fibronectin in the tissue samples treated with the test formulation was significantly lower in comparison to the untreated tissues ($p < 0.05$), for both cell extracts and growth media, on days 2 and 5. These results are consistent with Figures 2 and 3 which show a thinner epidermis, as a result of decreased collagen and fibronectin, for the tissues treated with Pentoxifylline 10% Topical Cream (XemaTop™), in comparison to the control tissues.

Conclusions:

The *in vitro* psoriasis tissue model is a valuable tool to evaluate the effect of topical formulations in psoriasis. The inhibition of collagen, fibronectin and the inflammatory cytokines (IL-1β, IL-6 and GM-CSF), following *in vitro* application of PCCA Formula 11934, suggests that Pentoxifylline 10% in XemaTop is likely to attenuate the inflammatory response and cellular proliferation associated with psoriasis *in vivo*. 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:

1. Yoshinaga, Y., Higaki, M., Terajima, S. *et al.* (1995) 'Detection of inflammatory cytokines in psoriatic skin'. *Archives of Dermatological Research*, 287 (2), 158-64.
2. Zargari, O. (2008) 'Pentoxifylline: A drug with wide spectrum applications in dermatology'. *Dermatology Online Journal*, 14 (11): 2.

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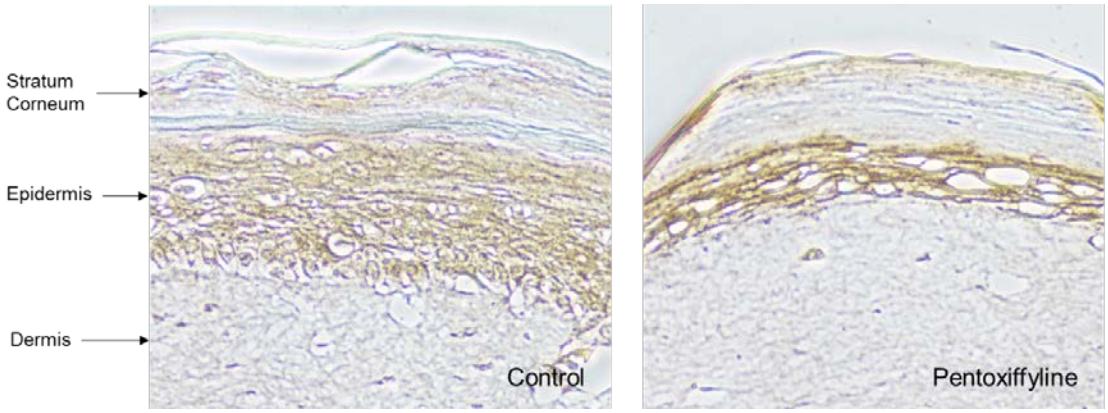
Table 1. *Mean concentration of IL-1β, IL-6 and GM-CSF detected* in vitro *following application of PCCA Formula 12374*

Test Formulation	Mean IL1β (pg/mL) ± SD		Mean IL-6 (pg/mL) ± SD		Mean GM-CSF (pg/mL) ± SD	
	Day 2	Day 5	Day 2	Day 5	Day 2	Day 5
Negative control (untreated tissues)	98.16 ± 2.78	80.09 ± 4.87	194.13 ± 17.87	204.75 ± 6.97	8.67 ± 0.93	8.61 ± 1.15
Pentoxifylline 10% Topical Cream (XemaTop™)	104.34 ± 6.49	63.96 ± 1.94	74.95 ± 17.93	11.25 ± 3.28	2.25 ± 0.39	0.36 ± 0.25
P-value	*0.131*	*0.001*	*8.16E^{-05}*	*4.16E^{-09}*	*1.46E^{-05}*	8.00E^{-06}

Test Formulation	Mean Pro-Collagen Type I Cell extracts (pg/mL) ± SD Growth media (ng/mL) ± SD		Mean Fibronectin Cell extracts (pg/mL) ± SD Growth media (ng/mL) ± SD	
	Day 2	Day 5	Day 2	Day 5
Negative control (untreated tissues)	118.64 ± 6.00 27.55 ± 3.47	120.99 ± 6.04 46.51 ± 8.48	3636.08 ± 127.79 143.61 ± 15.39	3023.24 ± 17.21 260.19 ± 17.00
Pentoxifylline 10% Topical Cream (XemaTop™)	49.44 ± 2.47 *0.004* 8.94 ± 0.69	60.62 ± 6.40 *0.010* 4.49 ± 0.89	1402.89 ± 160.89 *0.004* 89.54 ± 13.38	1858.89 ± 208.56 *0.016* 91.88 ± 12.59
P-value	*4.36E^{-05}*	6.28E^{-05}	*0.002*	3.91E^{-06}

Table 2. *Mean concentration of pro-collagen type I and fibronectin detected* in vitro *following application of PCCA Formula 12374*

Figure 2. *Immuno-histochemical staining of collagen (brown) following application of PCCA Formula 12374 for 2 days. Digital images were taken at 10x magnification.*



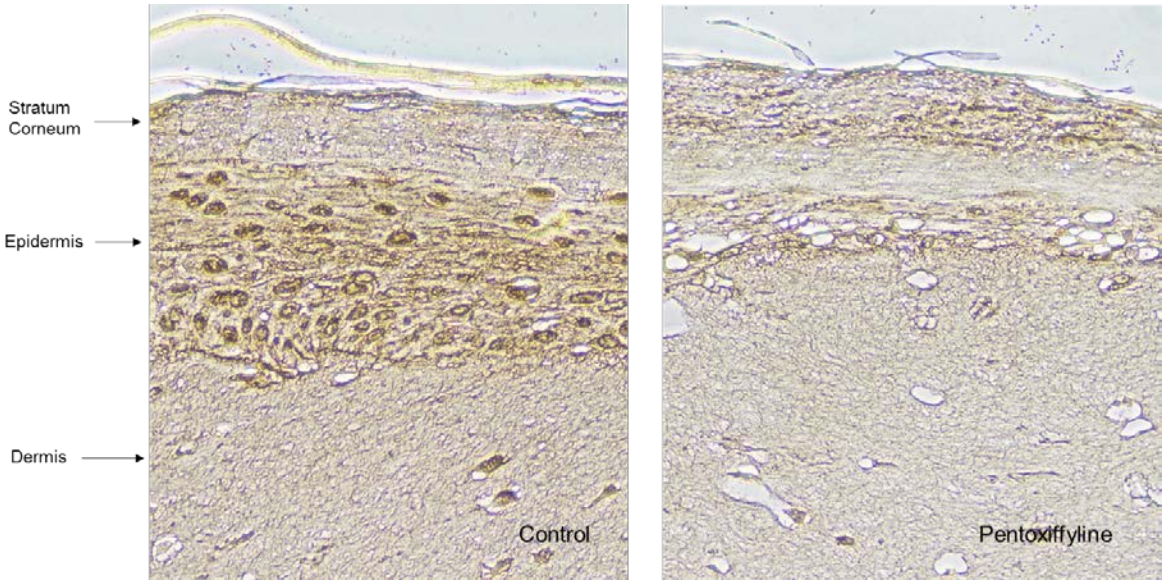


Figure 3. *Immuno-histochemical staining of fibronectin (brown) following application of PCCA Formula 12374 for 2 days. Digital images were taken at 10x magnification.*