Cytokine induction in the 3D EpiDerm™ skin model used as an *in vitro* preclinical screening tool for formulations with anti-inflammatory action

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ABSTRACT

Abnormal cytokine profiles represent the hallmark of inflammatory skin conditions such as psoriasis, acne, atopic dermatitis, irritant and allergic contact dermatitis. Damage to skin keratinocytes induces the release of primary cytokine interleukin (IL)-1α which further stimulates the release of secondary cytokines (e.g., IL-8) involved in the mediation of inflammatory reactions. Animal models have been historically used to assess the potency of formulations designed to intervene in the inflammatory cascade. In recent years, *in vitro* testing methods based on three-dimensional (3D) reconstructed skin equivalents became a reliable, rapid tool to screen actives and formulations for efficacy claims, including potential anti-inflammatory action. Here we present data generated in a novel *in vitro* assay based on the EpiDerm™ Human Cell Construct (MatTek Corporation). The EpiDerm™ tissues were exposed topically for 6 hours to materials intended to counteract the inflammation induced by phorbol-12-myristate 13-acetate (PMA) added to the culture media. Two different ingredients with known anti-inflammatory activity formulated as creams were evaluated (OTC-class 7 low potency, and Rx-class 2 high potency, formulated for augmented penetration). The low potency active was also tested as a spray along with an alcohol-based hand sanitizer. To avoid over-prediction of the irritation, the alcohol-based formulations were applied to the tissues at a reduced dosing volume of 30 µL, while the creams were applied as 100 µL doses. The cytokines analyzed were IL-1α and IL-8 (released in the culture media and in the lysed tissues). Our data showed that IL-1α analyzed in the lysed tissues and IL-8 analyzed in the culture media were reliable indicators of anti-inflammatory actions for the materials tested. Both cytokine indicators showed that the Rx cream formulated for augmented penetration was the most effective of the creams in reducing the cytokines' levels, thus supporting the class 2 high potency. Furthermore, the class 7 active formulated as a spray had a stronger anti-inflammatory action compared to its cream counterpart despite the reduced dosing volume. Our data support the potential use of the Rx class 2 cream and the OTC class 7 spray as reference materials for screening formulations investigated for anti-inflammatory action.

INTRODUCTION

Manufacturers of actives or formulations with potential anti-inflammatory action are in need of a rapid, reliable and relevant *in vitro* assay to be used as a screening tool before initiation of clinical testing. The assay we designed is making advances to fill that gap and provides data to support the use of reconstructed skin models and cytokine endpoints to interpret the anti-inflammatory action of various classes of products. We tested several types of anti-inflammatory products (pharmaceuticals and personal care) of different formulation types (cream and spray) that were applied topically to the EpiDerm™ tissues to counteract the inflammation induced by addition of PMA into the culture media (**Figure 1**).

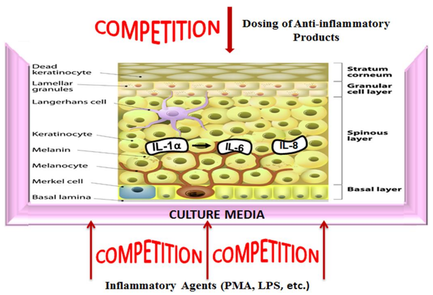

Figure 1. Overview of the test system

Upon the initiation of the inflammatory pathways by PMA, the keratinocytes synthesize pro-inflammatory cytokines (IL-1α), IL-8, and IL-6, etc. that mediate the primary contact irritancy reactions in the skin [1] captured *in vitro* by the EpiDerm™-based test system (**Figure 1**). Our data showed that the analysis of the compartmentalized cytokines synthesis (IL-1α) and secretion (IL-8) is a reliable predictor of the anti-inflammatory action of various compounds intended for human skin application.

MATERIALS AND METHODS

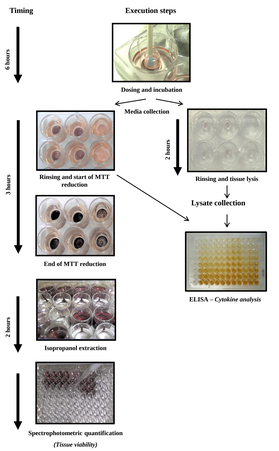

Figure 2. Overview of the *in vitro* assay

- **Test materials:**
  - anti-inflammatory OTC-class 7 low potency pharmaceutical product (cream and spray)
  - anti-inflammatory RX-class 2 high potency pharmaceutical product (cream formulated for augmented penetration)
  - hand sanitizer commercially available
- **Test system**: the EpiDerm™ model (MatTek Corporation) consisting of normal, human-derived keratinocytes
- **Inflammatory agent**: 10 µg/mL PMA prepared in EtOH
- **Assay controls**:
  - negative: 0.9% Saline
  - positive: OTC-class 7 low potency product (cream)
  - negative for inflammatory agent: EtOH at final concentration of 0.5% in the culture media
- **Dosing volumes**:
  - 100 µL for creams
  - 30 µL for alcohol-based formulations
- **Endpoints:**
  - tissue viability (%) – MTT assay
  - IL-1α, IL-8 — Analyzed in culture media and tissue lysates

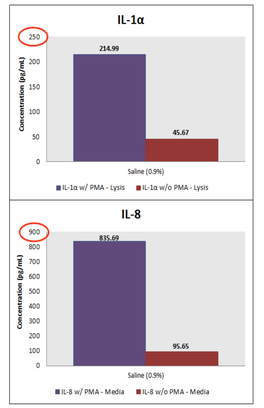

Figure 3. Induction of cytokine expression (IL-1α, IL-8) by PMA in tissues treated with the assay negative control (0.9% Saline)

RESULTS

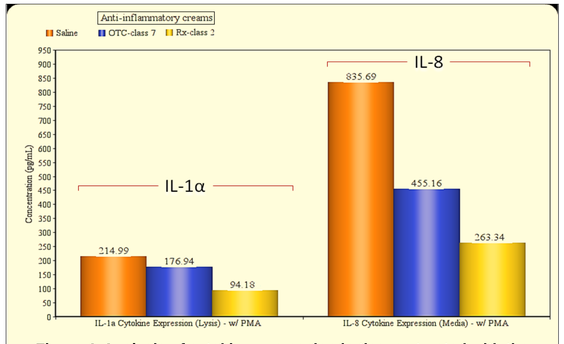

Figure 4. Analysis of cytokine expression in tissues treated with the anti-inflammatory products formulated as creams

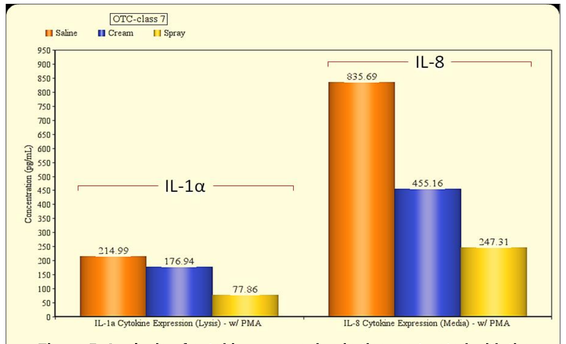

Figure 5. Analysis of cytokine expression in tissues treated with the OTC-class 7 low potency products

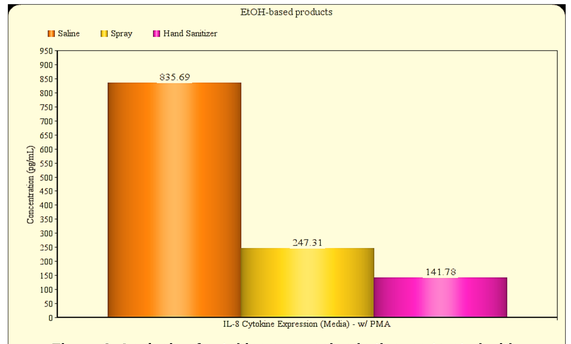

Figure 6. Analysis of cytokine expression in tissues treated with alcohol-based products

The EpiDerm™ tissues were treated topically with each test material; PMA was added into the culture media. The expression of IL-1α was analyzed in the lysates of tissues cultured in media containing PMA, while IL-8 was analyzed in the culture media (secretion). Tissue viability for all treated tissues was >80%.

- The OTC-class 7 (low potency) and Rx-class 2 (high potency, formulated for enhanced penetration) products were tested.
- Both cytokine indicators showed that the Rx-class 2 cream was the most effective of the two creams in reducing the levels of the inflammatory cytokines.
- The results support the high potency efficacy claim of the Rx-class 2 cream over the OTC-class 7 (low potency cream).
- The OTC-class 7 (low potency) products were applied topically at 100 µL dose for the cream, and as 30 µL dose for the spray, respectively.
- The reduced volume for the spray formulation was based on our preliminary data indicating that higher doses of alcohol-containing products could lead to possible over-prediction of the irritation potential.
- Both cytokine indicators showed that the OTC-class 7 product formulated as spray had a stronger anti-inflammatory action compared to its cream counterpart despite the reduced dosing volume. The anti-inflammatory potential of the spray was comparable to the Rx-class 2 product (**Figure 4**).
- Our results seem to indicate that the bioavailability of the anti-inflammatory ingredient formulated as spray could be increased by the presence of the alcohol.
- The OTC-class 7 (low potency) product formulated as spray and a commercially available alcohol-based hand sanitizer were tested as 30 µL doses.
- The alcohol content of the OTC-class 7 spray was 45%, while the alcohol content of the hand sanitizer was 70%.
- The analysis of IL-8 in the culture media indicated that the hand sanitizer had a more pronounced anti-inflammatory action on the tissues compared to the OTC-class 7 spray.
- Our preliminary results indicate that the alcohol content could be a contributing factor to the anti-inflammatory action of pharmaceutical sprays or sanitizers with antimicrobial claims.

CONCLUSIONS AND FUTURE CONSIDERATIONS

1. The efficacy testing for products with anti-inflammatory claims can be conducted using *in vivo* test systems [2] and ultimately by human clinical studies. The reliability of the animal test system has been re-assessed recently [3], bringing into attention once again the need for a fast, reliable and relevant *in vitro* assay that can be used as a pre-clinical screening tool.
2. Several *in vitro* assay designs exist for quick pre-clinical testing [4, 5] that show promise addressing industry's need for a testing strategy to qualify prototypes for subsequent clinical studies investigating anti-inflammatory claims.
3. The *in vitro* assay used in our study analyzed the reduction of cytokine expression in the EpiDerm™ reconstructed skin model under PMA-induced inflammatory state after treatment with products with anti-inflammatory action either clinically demonstrated (OTC and Rx pharmaceutical products) or unknown (hand sanitizer).
4. Our data demonstrated that the compartmentalized cytokine expression (IL-1α – tissues lysate, and IL-8 – secreted in culture media) is a reliable indicator of inflammation induced by PMA and subsequently of the anti-inflammatory action of the products tested.
5. Our results support the use of the Rx-class 2 cream and the OTC-class 7 spray as reference materials when screening formulations investigated for anti-inflammatory action.
6. Several studies advanced involvement of alcohols in modulating inflammatory pathways [6-8]. The preliminary results we report for the hand sanitizer open the discussion of a possible anti-inflammatory effect of alcohol-based personal care products.
7. Our future plans will focus on screening a diverse range of actives and formulations in order to expand the applicability domain of the *in vitro* assay and will also consider widening the cytokine profiling.

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