Optimization of an in vitro pre-clinical screening tool for evaluation of anti-inflammatory activity of raw ingredients and finished products

Abstract

In vitro testing methods based on three-dimensional (3D) reconstructed skin equivalents are reliable, rapid tools frequently used to screen actives and formulations for efficacy evaluation, including potential anti-inflammatory activity. Presented here are data generated using an established in vitro assay based on the Epiderm™ Human Cell Construct (MatTek Corporation, Ashland, MA, USA) that was optimized to reduce the time of inflammatory cascade induction that may be more appropriate for the screening of certain actives or product lines. In the standard assay, the Epiderm™ tissues are exposed topically for 6 hours to materials intended to counteract the inflammation induced by phorbol-12-myristate-13-acetate (PMA) (100 nM) and to the culture media. The tissue viability and interleukin (IL)-8 secretion in collected culture media are the assay endpoints evaluated at the completion of the exposure time. To accommodate the evaluation of products that need to be tested for short exposure times to the tissues depending on consumer use or other considerations, the assay was optimized in two phases as further described. In the first phase, the kinetics of inflammatory events was investigated to determine if a shorter exposure of the tissues to PMA would induce significant expression of the IL-8 to allow reliable data interpretations. In these experiments, unexposed Epiderm™ tissues were first pre-incubated overnight in media without PMA. Then 3 sets of duplicate tissues were transferred in media containing PMA for exposure times varying from 1 to 6 hours followed by 5 to 0 hours post-exposure to media without PMA, respectively. The culture media was collected at the completion of the exposure to PMA and then again every hour until the end of the post-exposure in media without PMA, for all groups; this allowed for the evaluation of IL-8 signal depletion in the absence of the induction agent. The results showed a time-dependent reduction of IL-8 expression by the tissues exposed to a time-dependent reduction of the cytokine signal in the post-exposure period. Of the treatment groups included in this phase, one incubated in PMA-containing media for 3 hours followed by a 3 hours post-exposure without PMA showed a significant reduction of the IL-8 to allow for reliable data analysis and it was selected for the second, confirmatory phase of the experiments. In this second phase, the unexposed tissues were considered to be the assay negative control, while a commercially available Class 2 (high potency) anti-inflammatory cream was used as the assay positive control. These experiments confirmed the performance of the optimized assay based on the control data. Our data demonstrate that a shorter 3 hours exposure to PMA induces a significant production of IL-8 in barrier inflammatory state to allow for data interpretation and ranking of products regarding their anti-inflammatory potential. This optimized protocol not only shortens the assay length with similar results to the standard one but also accommodates testing of products that require a customized testing strategy that may be based on shorter exposure times to the inflammatory agent and/or protocols investigated.

Materials & Methods

In the optimized protocol, the tissues exposed to PMA successfully induced the expression of IL-8 and IL-10, respectively phase, the expression of these cytokines were differentially expressed either without PMA or with PMA treatment. The optimized protocol was conducted by first conducting a preventive treatment to evaluate the anti-inflammatory activity of the product followed by a time-dependent treatment of the cytokine signal in the post-exposure period. Of the treatment groups included in this phase, one incubated in PMA-containing media for 3 hours followed by a 3 hours post-exposure without PMA showed a significant reduction of the IL-8 to allow for reliable data analysis and it was selected for the second, confirmatory phase of the experiments. In this second phase, the unexposed tissues were considered to be the assay negative control, while a commercially available Class 2 (high potency) anti-inflammatory cream was used as the assay positive control. These experiments confirmed the performance of the optimized assay based on the control data. Our data demonstrate that a shorter 3 hours exposure to PMA induces a significant production of IL-8 in barrier inflammatory state to allow for data interpretation and ranking of products regarding their anti-inflammatory potential. This optimized protocol not only shortens the assay length with similar results to the standard one but also accommodates testing of products that require a customized testing strategy that may be based on shorter exposure times to the inflammatory agent and/or protocols investigated.

Standard Protocol

<table>
<thead>
<tr>
<th>Time</th>
<th>Media</th>
<th>Treatment</th>
<th>Control</th>
<th>Incubation</th>
<th>Without Media</th>
<th>Media Only</th>
<th>Combined Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>Media 1</td>
<td>PMA 100 nM</td>
<td>Positive</td>
<td>1 hr incubation</td>
<td>0.9% Saline</td>
<td>PMA 100 nM</td>
<td>PMA 100 nM + 0.9% Saline</td>
</tr>
<tr>
<td>1 hr</td>
<td>Media 2</td>
<td>PMA 100 nM</td>
<td>Negative</td>
<td>1 hr incubation</td>
<td>PMA 100 nM</td>
<td>0.9% Saline</td>
<td>PMA 100 nM + 0.9% Saline</td>
</tr>
</tbody>
</table>

Optimized Protocol

<table>
<thead>
<tr>
<th>Time</th>
<th>Media</th>
<th>Treatment</th>
<th>Control</th>
<th>Incubation</th>
<th>Without Media</th>
<th>Media Only</th>
<th>Combined Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>Media 1</td>
<td>PMA 100 nM</td>
<td>Positive</td>
<td>3 hr incubation</td>
<td>0.9% Saline</td>
<td>PMA 100 nM</td>
<td>PMA 100 nM + 0.9% Saline</td>
</tr>
<tr>
<td>1 hr</td>
<td>Media 2</td>
<td>PMA 100 nM</td>
<td>Negative</td>
<td>3 hr incubation</td>
<td>PMA 100 nM</td>
<td>0.9% Saline</td>
<td>PMA 100 nM + 0.9% Saline</td>
</tr>
</tbody>
</table>

Results

Question 2: What is the kinetics profile of IL-8 production after the incubation in the presence of PMA?

Answer 2: The kinetics profile of IL-8 production showed a similar trend to the most common use by consumers (Fig. 2).

Question 3: Which exposure group provides the most consistent and positive control for IL-8 production?

Answer 3: Group 3 (3G) showed a significant reduction of IL-8 after 6 hours exposure to PMA and progressive, hourly time-dependent reduction of the cytokine expression in the post-exposure period.

Conclusions & Future Directions

Based on the optimized protocol, the tissues exposed to PMA successfully induced the expression of IL-8 and IL-10, respectively phase, the expression of these cytokines were differentially expressed either without PMA or with PMA treatment. The optimized protocol was conducted by first conducting a preventive treatment to evaluate the anti-inflammatory activity of the product followed by a time-dependent treatment of the cytokine signal in the post-exposure period. Of the treatment groups included in this phase, one incubated in PMA-containing media for 3 hours followed by a 3 hours post-exposure without PMA showed a significant reduction of the IL-8 to allow for reliable data analysis and it was selected for the second, confirmatory phase of the experiments. In this second phase, the unexposed tissues were considered to be the assay negative control, while a commercially available Class 2 (high potency) anti-inflammatory cream was used as the assay positive control. These experiments confirmed the performance of the optimized assay based on the control data. Our data demonstrate that a shorter 3 hours exposure to PMA induces a significant production of IL-8 in barrier inflammatory state to allow for data interpretation and ranking of products regarding their anti-inflammatory potential. This optimized protocol not only shortens the assay length with similar results to the standard one but also accommodates testing of products that require a customized testing strategy that may be based on shorter exposure times to the inflammatory agent and/or protocols investigated.

References


