Evaluation of the soothing activity of Stearyl Glycyrrhetinate

Abstract
Stearyl Glycyrrhetinate is a derivative of Glycyrrhetinic Acid, known in the pharmaceutical and cosmetic industry for its soothing properties. The aim of this study is, after confirming the soothing activity of Glycyrrhetinic Acid with an In Vivo test vs. placebo, to evaluate, again with an In Vitro test, its speed of action, based on a higher bio-availability of the esterified active. The In Vivo test has been carried out using co-culture systems of human keratinocytes and fibroblasts.

INTRODUCTION

Glycyrrhetinic Acid and Glycyrrhizic Acid are specific compounds isolated from wild licorice plants. Licorice (Glycyrrhiza glabra L.) has a long history of medicinal use in Europe and Asia and has been widely used in traditional Chinese medicine. It is reported to be effective in the treatment of peptic ulcer disease, constipation, skin damage, coughs and other ailments (1,2).

A large number of components have been isolated from licorice over the years, in particular glycyrrhizic acid, which is normally considered to be the main biologically active component. Glycyrrhizic Acid is a conjugate of Glycyrrhetinic Acid and two molecules of glucuronic acid, a carbohydrate. Stearyl Glycyrrhetinate is the salt and ester of Glycyrrhetinic Acid (Figure 1).

The anti-inflammatory effect of Glycyrrhizic Acid has been studied chemically and pharmacologically over the years. Glycyrrhetinic Acid is a pentacyclic triterpenoid. The structure of Glycyrrhetinic Acid is similar to that of cortisone. This may be the basis for licorice’s anti-inflammatory action (3,4).

In Glycyrrhetinic Acid, the functional group [R] is a hydroxyl group. Stearyl Glycyrrhetinate is the ester derivative of glycyrrhetinic acid. The reaction scheme is following: RCOOH + ROH D RCOOR + H2O

Stearyl Glycyrrhetinate has a highly oil-soluble saturated molecular group structure. It dissolves in a lipidic environment better than Glycyrrhetinic Acid. It has good compatibility, it easily solvates in several types of lipids and alcohols. Compared to glycyrrhetinic acid, Stearyl Glycyrrhetinate has a low melting point.

In cosmetic and pharma industries, it has a long history of use; it has been used to improve anti-viral effects, reduce inflammation, prevent allergies, cleanse the skin, provide skin whitening and to assist with sun protection.

The aims of this study are to show that Stearyl Glycyrrhetinate maintains the soothing properties of Glycyrrhizic Acid and that Stearyl Glycerrhetinate has a better overall attributes compared to Glycyrrhetinic Acid (4).

IN VITRO STUDY

Material and Methods

Human Keratinocytes (5,6,7)

The human keratinocytes were seeded (100,000 cells/ml Dulbecco’s high glucose supplemented with 10% foetal bovine serum) and cultured for ten days, to reach the confluence of 100% of semi permeable inserts (pore size of 0.6 cm and 0.4μm - Falcon). During this time period, after having reached confluence, the culture medium was removed and the cells were left in contact with the air to improve the compactness of the multi-layer of cells. Indeed the air is considered a harmful agent that
DISCUSSION

In the present study, the experimental model adopted has allowed us to confirm not only the soothing activity of Glycyrrhetinic acid contained in Stearyl Glycyrrhetinate, but also to compare the power of penetration of the two forms of the active principle, through the barrier formed by keratinocytes that have grown forming a multi stratified monolayer (see Figure 3). The results obtained confirm a stronger soothing activity of Stearyl Glycyrrhetinate than Glycyrrhetinic acid. Stearyl Glycyrrhetinate reduces the secretion of IL8 and IL6 by 95 and 36% after 120 minutes of exposure, while glycyrrhetinic acid reduces the interleukins secretion by 83% and 15% respectively.

Analyzing the secretion of the two cytokines in time we observed how 15 minutes is sufficient for Stearyl Glycyrrhetinate to pass through the layer of keratinocytes from the upper chamber to the lower one and to act as a soothing agent on fibroblasts sensitized with LPS, reducing the synthesis of IL6 and IL8. The results also show a good inhibition of the release of IL8 after 15 minutes of exposure, however, the amount of acid migrated into the lower chamber is not sufficient to produce significant inhibition of the release of IL6 only after 120 minutes of exposure.

The results show therefore a higher bioavailability of Stearyl Glycyrrhetinate. It can reach the inflamed site quicker than the acid and act faster against inflammation.

IN VIVO STUDY

According to the results obtained with the in vitro testing, the soothing effects were then tested and confirmed by vivo test. “Controlled evaluation of soothing and repairing effect of a cosmetic product with Pantrofina® Beta (INCI: Stearyl Glycyrrhetinate) on the damage caused by a chemical irritant agent [SLS]” was the title of the test.

Material and Methods

The aim of the in vivo study was to evaluate the soothing and repairing effect of a cosmetic product against skin alterations caused by an irritant agent of chemical nature.

The test product [mean of the values obtained from two treated areas] was compared with both an untreated skin area [mean of the values obtained from two untreated areas] and a placebo treated skin area (mean of the values obtained from two placebo treated areas). The test was carried out on a panel of 10 volunteers; subjects were recruited to take part in the test.
Figure 3. In vitro testing results of Stearyl Glycyrrhetinate

### INHIBITION OF IL8

<table>
<thead>
<tr>
<th>Soothing Activity</th>
<th>IL6 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Pan β</td>
</tr>
<tr>
<td>15 min</td>
<td>443,56</td>
</tr>
<tr>
<td>30 min</td>
<td>382,31</td>
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<tr>
<td>60 min</td>
<td>425,23</td>
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<td>120 min</td>
<td>420,44</td>
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<td>Pos Control</td>
<td>660,13</td>
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### INHIBITION OF IL6

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<th>% IL6 Reduction</th>
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<tr>
<td>15 min</td>
<td>32,81</td>
</tr>
<tr>
<td>30 min</td>
<td>42,08</td>
</tr>
<tr>
<td>60 min</td>
<td>35,58</td>
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<td>120 min</td>
<td>36,31</td>
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<table>
<thead>
<tr>
<th>Soothing Activity</th>
<th>IL6 pg/ml</th>
</tr>
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<tbody>
<tr>
<td>Time</td>
<td>Pan β</td>
</tr>
<tr>
<td>15 min</td>
<td>65,7</td>
</tr>
<tr>
<td>30 min</td>
<td>26,6</td>
</tr>
<tr>
<td>60 min</td>
<td>29</td>
</tr>
<tr>
<td>120 min</td>
<td>43</td>
</tr>
<tr>
<td>Pos Control</td>
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<table>
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<th>Soothing Activity</th>
<th>% IL6 Reduction</th>
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<td>Time</td>
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<td>15 min</td>
<td>92,90</td>
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<td>30 min</td>
<td>97,12</td>
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<td>60 min</td>
<td>96,82</td>
</tr>
<tr>
<td>120 min</td>
<td>95,32</td>
</tr>
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</table>
The study was carried out as follows:

- Induction of the damage: to each volunteer was applied SLS by 6 Finn Chambers, to induce irritation. Six chambers of the patch were filled with 2% SLS aqueous solution and applied on backs of the volunteers. 24 hours after application, the Finn Chambers were removed.
- The instrumental evaluations were acquired before (T0), after the damage induction (24 hours after Finn Chambers application, 15 minutes after their removal), and after the product’s application (30 minutes, 1, 2, 24 hours), T30min, T1h, T2h, T24h.
- Trans epidermal water loss was measured by means of the internationally recognized TEWAMETER® method. The instrument used was a Tewameter 300® (Courage+Khazaka, electronic GmbH).
- Burning, mechanical pressure, heat, chemicals can induce the manifestation of skin redness (erythema)

The MEXAMETER 18 specifically measures the hemoglobin content (erythema) in the skin. The measurement is based on the absorption principle. The special probe of the MEXAMETER 18 emits light of three defined wavelengths. A receiver measures the light reflected by the skin.

**DISCUSSION**

The Figure 4 shows the mean percentage variations vs SLS of the ERYTHEMA INDEX obtained after product application compared with both mean percentage variations vs SLS obtained in the control area (CTR) and in the placebo treated area.

As it is possible to notice the product with Pantrofina β induces, at preliminary level, a reduction of the erythemal reaction, induced by SLS, at all experimental checks. The statistical analysis shows that the product effect is greater than the one obtained:
- with UNTREATED AREA (at T30min, T1h, T2h, T24h);
- with PLACEBO TREATED AREA (at T1h, T2h, T24h).

As it is possible to notice, the product with Pantrofina β shows, at preliminary level, a reduction of the reaction, induced by SLS. The product application determines a reduction of the trans epidermal water loss greater than the one obtained with untreated area (CTR), thus accelerating the normal physiological recovery process. (fig. 5)

The statistical analysis shows that the product effect is greater than the one obtained:
- with UNTREATED AREA (at T30min, T1h, T2h, T24h);
- with PLACEBO TREATED AREA (at T2h, T24h).
CONCLUSION
Both in vitro and in vivo testing confirms the soothing activity of Stearyl Glycyrrhetinate in cosmetic applications.

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Prevention and treatment are the strategies used to reduce the age-related effects on our skin. Anti-ageing technologies refer to the treatments used to improve the look and condition of the skin and include the devices and methodologies applied to measure these benefits. The sophistication and advancements in skin and cosmetic science are heightening expectations of the new technologies being introduced into anti-ageing skin care.

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3. Impact of consumers and regulations
4. Anti-ageing measurements and treatment

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