Topically applied antioxidants protect the skin from chemical-induced irritations

Introduction
Due to former scientific findings, reactive oxygen species (ROS) do considerably contribute to the development of allergic contact dermatitis. It has been shown that antioxidants such as N-acetylcysteine (NAC) and vitamin E (tocopherol) may counteract the development of irritant as well as specific contact hypersensitivity reactions. As already shown in previous studies, several antioxidants protect each other and they form an antioxidant network inside of the skin. The aim of the present study was to evaluate the potential of topically applied antioxidants to prevent irritant contact dermatitis. For this purpose and with the help of the repetitive washing test, the effect of a cream containing a synergistic effects showing combination of the three antioxidants Reseda luteola extract (= luteolin, one of the most powerful natural antioxidants with anti-inflammatory characteristics), tocopherol (vitamin E) and ubiquinone (coenzyme Q10), the respective vehicle (lamellar cream base without active principles) and the reference (Excipial Protect®, an established skin protection cream), on a sodium lauryl sulfate-induced irritant contact dermatitis was examined.

In order to assess the impact on the skin’s barrier function and its inflammation condition, three readout parameters were chosen: a) the hydration of the skin’s horny layer (corneum), b) the blood flow inside of the skin (Laser-Doppler flowmetry) and c) the transepidermal water loss (TEWL).

It has been shown that the topical application of antioxidants may protect the skin from chemical-induced irritation. In comparison to the vehicle and the reference, the high radical protection factor (RPF) of the cream containing active principles points out that reactive oxygen species (ROS), at least in part, contribute to the development of irritant contact dermatitis.

Implementation
In order to evoke a standardized and therefore comparable irritant contact dermatitis, the repetitive washing test was performed on 25 healthy test persons. In short washings with 0,01 M sodium lauryl sulfate, the test was performed on four test areas placed on the inner forearms of the test persons three times a day on seven consecutive days. Fifteen minutes prior to the washings, 200 μl of the test creams were topically applied to three of the four test areas, whereas one of them remained untreated.

The hydration of the skin’s horny layer (corneum), the transepidermal water loss (TEWL) and the blood flow inside of the skin were chosen to be the readout parameters for the skin’s barrier function and its inflammation. They were evaluated with biotechnological and commonly used methods. Three instrumental measurements were made: before the first treatment (T0), after three days (T3) and after seven days (T7).

The cream containing antioxidants (verum) was compared to the respective cream basis (vehicle) and to the reference, a well-known protection cream (Excipial Protect®, Spirig Pharma, Egerkingen, Switzerland). The cream with antioxidants (verum) contained Reseda luteola extract (luteolin), vitamin E and ubiquinone as active principle, whereas aluminium chlorohydrate is the one of the reference. In order to evaluate the antioxidant properties of the test preparations, their radical protection factor (RPF) was determined by being measured with the electron paramagnetic resonance spectroscopy.
Results

a) Corneum hydration

The repetitive washing test led to a statistically significant loss of corneum hydration in untreated skin. On day 3 as well as on day 7, the dehydration was moderated by the reference (Excipial Protect®) and by the vehicle, whereas this effect was more distinct when the reference has been applied. Despite repetitive washings, the verum was the only formulation that led to an improvement of corneum hydration on both observation times.

b) Laser-Doppler flowmetry (LDF)

Moreover, the repetitive washing test led to an increasing inflammation reaction of untreated skin, which was documented as an increase in cutaneous blood flow. All of the three test formulations counteracted this effect. The verum prevented the increase of cutaneous blood flow completely on day 3 and reduced it most distinctly on day 7. On day 3, the verum and the reference could be differentiated statistically.

Figure 1: Results of the repetitive washing test. Instrumental measurements were made before the first treatment (T0), after three days (T3) and after seven days (T7). The mean and standard deviation of each group are included. NS = not significant

a) Corneum hydration

b) Laser-Doppler flowmetry (LDF)
c) Transepidermal water loss (TEWL)

The repetitive washing test led to an increase in transepidermal water loss in untreated skin which was particularly distinct on day 7. The reference and the verum minimized the increase in TEWL and cannot be differentiated statistically. The vehicle did not have a reducing effect on the TEWL, neither on day 3 nor on day 7.

d) Radical protection factor (RPF)

The determination of the radical protection factor has clearly shown that the RPF of the verum is almost 19-times higher than the RPF of the vehicle and the reference.

<table>
<thead>
<tr>
<th>formulation</th>
<th>RPF ($10^{14}$ radicals/mg)</th>
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</thead>
<tbody>
<tr>
<td>reference</td>
<td>$15 \pm 1$</td>
</tr>
<tr>
<td>vehicle</td>
<td>$17 \pm 1$</td>
</tr>
<tr>
<td>verum</td>
<td>$320 \pm 16$</td>
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</tbody>
</table>

**Conclusion**

Topically applied antioxidants may protect the skin from chemical-induced irritation. As reactive oxygen species contribute considerably to the development of allergic contact dermatitis, the radical protection factor (RPF) can be an assessment criterion for the used formulations. In the present study, all objects of comparison had a lower radical protection factor (RPF) than the verum. Therefore, this cream containing luteolin with an active principle based on the combination of three antioxidants and a very high radical protection factor (RPF) is clearly superior to the vehicle and the reference, the established skin protection cream named Excipial Protect®.