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Stimulating and Protecting Skin Immunity to Decrease UV-Induced Skin Erythema
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Introduction

Skin is permanently exposed to stress from the external environment. In order to defend itself and to increase its repair capacity, skin possesses molecules that are part of the innate immunity system. These molecules are expressed by the keratinocytes and also present on Langerhans cells. They are highly conserved through evolution and represent the first line of defense against foreign antigens and environmental stress. Although during the early response these molecules act locally, they may trigger eventually a more systemic immune response if the aggression can not be resolved rapidly. These molecules also called innate immunity markers can be considered, together with the Langerhans cells, the skin immune sentinels (1) making sure that a pro-inflammatory aggression is detected and controlled (1-4). Among the skin immunity markers we can list anti-microbial peptides like cathelicidins and defensins that directly kill invasive microbes (5, 6); heme oxygenase 1 (HO-1), involved as an anti-oxidant and wound healing agent (7, 8); S100 proteins, with both anti-microbial (9) and skin barrier properties (10); and Toll like receptor-2 with signaling function (10) but also important in anti-microbial defense (11, 12).

Unfortunately, external stress and in particular UV light can significantly reduce the innate immunity markers and the Langerhans cells (13-16), depriving the skin from its basic defenses and increasing the possibility of antigen-triggered inflammation.

With a reduced immune defense, the skin would be less reactive and unbalanced with the need of regaining its proper healthy balance and reactivity. The approach is then to stimulate innate immunity markers and Langerhans cells with the goal to re-balance an under-reacting skin. Doing so, we believe we can reduce upcoming external aggressions with associated inflammation and provide therefore a long term skin soothing. In order to stimulate innate immunity and early defenses we have selected ingredients that have been described in the scientific literature for their potential in soothing skin irritation through modulation of the immune system. A bioactive complex was formulated, con-

Abstract

Skin defense and reactivity involve production by keratinocytes of innate immunity proteins. These proteins, also expressed on skin Langerhans cells (the immunity sentinels), help the skin reacting to environmental aggressions and repairing damage. A complex of natural ingredients was formulated to stimulate and to protect skin’s immunity. A combination of middle weight polysaccharides from Tamarindus and glycoside Stevioside (trade name: Unisooth ST-32) was able to stimulate in human keratinocytes, markers of innate immunity such as Defensin beta 4 (+127%) and Heme Oxygenase 1 (+51%) and to protect Langerhans cells from UV-induced down regulation in human skin (63% protection). Furthermore, the complex formulated at 3%, reduced significantly UV-induced erythema in human volunteers (n=25) by 59% and Trans Epidermal Water Loss (TEWL) by 68% when compared to a placebo. In conclusion, it is possible to significantly prevent and reduce UV-induced irritation by boosting skin’s immunity with a combination of natural ingredients. The complex is suitable for day, 24 hours and sensitive skin application products.
taining a balanced blend of Tamarindus middle weight polysaccharides extracted from the plant’s seeds combined with the glycoside Stevioside, extracted from the Stevia leaves (trade name: Unisooth ST-32). Tamarindus Indica has been associated to anti-inflammatory properties in traditional medicine (17) and more recently its polysaccharides have shown soothing characteristics, being often compared to Aloe oligosaccharides for their efficacy (18, 19). Tamarindus polysaccharides have also shown the capacity to protect against UV-induced damage (20), suggesting its utilization as a UV protector.

Stevioside is a well known sweetener in the food industry, however its healthy benefits have been recently evidenced (21). In particular, its capacity as an immune modulator and anti-inflammatory characteristics have been studied (22, 23).

The hypothesis was then to test this complex to stimulate, in human keratinocytes, the innate immunity markers responsible for skin defense and repair and to protect, in human skin explants, Langerhans cells from UV-induced depletion (Fig. 1). Finally, the complex formulated at 3% in a water based gel was applied on human volunteers for reducing UV-induced erythema and trans-epidermal water loss (TEWL).

Materials and Methods

Normal human epidermal keratinocytes (NHEK) culture, treatment and analysis for innate immunity markers

NHEK at 3rd passage were seeded in 24-well plates in culture medium and incubated until confluence. The medium was then replaced by assay medium containing or not Stevioside at concentrations of 0.2%, 0.03%, and 0.001% or Tamarindus polysaccharides at concentration of 0.01% and left for 24 hours. All experimental conditions were performed in triplicate (n=3). At the end of the incubation, the cells were washed in phosphate buffered saline (PBS) solution and extracted for mRNA; mRNA was reverse-transcribed and genes for Cathelicidin, Defensin beta 4, Heme Oxygenase 1 (HO-1), S100 A7, Toll-like receptor-1, were analysed.

Skin explants culture, treatment and analysis for Langerhans cells

Skin explants from plastic surgery were treated topically with 20 mg/cm² of Unisooth ST-32 (3% in water) or a control reference (SPF30 solar cream), and incubated for 24 hours. Explants were then irradiated with a total dose of 1500 mJ/cm² UVB (SOL500 Sun Simulator with H2 filter). Non irradiated controls were kept in the dark. After irradiation, the skin explants were again treated with Unisooth ST-32 or the control reference and incubated for additional 24 hours. At the end of the incubation, punches were performed on each skin explant, tissue sectioned and incubated with an anti-CD1a-FITC antibody specifically recognizing Langerhans cells (LC). Observations were performed in epifluorescence with a Nikon E400 microscope and fluorescent LC were counted (n = 45/condition). Digital images were recorded with a Nikon DXM 1200F camera and Lucia 4.8 software.

Percentage of protection was calculated according to the following formula:

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\text{Protection} \% = \frac{\text{Positive Control (with UV)} - \text{Treatment + UV}}{\text{Positive Control (with UV)} - \text{Negative Control (without UV)}} \times 100
\]

Induction and measurement of Erythema and Trans-Epidermal Water Loss (TEWL) in human volunteers (double blind study)

Skin erythema was induced on human volunteers (n = 25) by exposure to UV light (UVA + UVB, solar spectrum). On each volunteer, the MED was determined by applying a pattern of radiation consisting in 6 doses of UV radiation. The radiation pattern was chosen based on the subject phototype. Once each individual MED was established, an erythematic reaction was induced in 9 skin areas of the volunteers back by using an UV irradiation corresponding to 1.25x MED. 6 hours before irradiation (T0), 2 mg/cm² product was applied on the back of the volunteers. 3 areas were treated with a placebo gel, 3 areas were treated with a water based gel containing 3% of Unisooth ST-32 and 3 areas were left untreated (irradiated CT). Skin redness (evaluated by Erythema index) and TEWL were measured 24 hours and 48 hours from T0. Comparison was made between all samples and to the untreated areas. Erythe-
ma index was measured using a Mexa-meter MX 18 (Courage+Khazaka, electronic GmbH), while TEWL was measured using a Tewameter 300 (Courage+Khazaka, electronic GmbH). Data were analyzed and expressed as % variation vs T0. Statistical significance was also calculated.

- **Statistical Analysis**

The inter-group comparisons were performed by Student’s T test.

- **Results**

**Stimulation of innate immunity markers in NHEK**

Treatment of NHEK with 0.01% Tamarindus polysaccharides did not bring any differences to innate immunity markers (not shown), while treatment of NHEK with different concentrations of Stevioside, increased the transcription of innate immunity markers (Cathelicidin, Defensin beta 4, S100 A7, Toll-like receptor 2 and Heme oxygenase 1) as shown in Fig. 2. This increase was dose dependent. In particular, Defensin beta 4 was strongly stimulated even at concentration of 0.03%.

**Protection of Langerhans cells in human skin explants**

In irradiated skin explants, the number of Langerhans cells in epidermis was significant lower compared to control non-irradiated explants. Treatment with Unisooth ST-32 at 3% significantly protected from UV-induced depletion Langerhans cells. The protection was 63% (p<0.001, Student’s T test). Immunofluorescence pictures are shown in Fig. 3. Topical treatment with control reference sun cream SPF30 protected the explants against UV-induced Langerhans cells depletion by 67% (data not shown).

**Reduction of UV-induced erythema and Trans-epidermal water loss (TEWL) in human volunteers**

As shown in Fig. 4, pre-treatment of human volunteers with Unisooth ST-32 at 3% in a water based gel, induced a reduction in UV-induced skin erythema of 48.7% and 58.8% when compared to a placebo treatment, after 24 hours and 48 hours from UV-irradiation, respectively. This decrease was statistically significant (p<0.001 vs placebo, Student’s T test). In the same volunteers, UV-induced skin trans-epidermal water loss (TEWL) was...
Reduced by the gel containing Unisooth ST-32 at 3%. The decrease was of 68.3% and 68.4% when compared to a placebo treatment, after 24 hours and 48 hours from UV-irradiation, respectively (Fig. 5). This decrease was statistically significant (p<0.001 vs placebo, Student’s T test). The reduction of both the erythema and the TEWL suggests that Unisooth ST-32 may not only have a soothing but also a healing effect.

**Conclusion**

Unisooth ST-32 stimulates synthesis of innate immunity markers and protects Langerhans cells. These effects suggest that Unisooth ST-32 can have immunostimulating and protecting properties. Since UV light and stress induce immunosuppression and reduced response to environmental aggressions (13-16), it is necessary to boost skin defenses mechanisms linked to innate immunity markers such as the anti-microbial peptides, repairing markers such as S100A7 and HO-1 and to protect Langerhans cells. The observed stimulation by Stevioside and Tamarindus polysaccharides of these mechanisms confirm the capacity of these ingredients to stimulate a mechanism of protection and repair that can be associated to a long term soothing. The effect on Langerhans cells was almost as good as an SPF30 cream used as a control reference (63% vs 67%, data not shown).

When Unisooth ST-32 was incorporated in a water based gel at concentration of 3% and applied topically on human volunteers before and after UV irradiation, it was able to significantly reduce the UV-induced erythema. The effect was visible already after 24 hours from UV irradiation and increased after 48 hours. Interestingly, Unisooth ST-32 was also able to dramatically decrease the trans-epidermal water loss (TEWL) induced by the UV irradiation. This effect was statistically significant and already impressive after 24 hours, while not increasing after 48 hours.

These data in vivo support the utilization of Unisooth ST-32 immuno-modulatory characteristics to reduce a UV-induced irritation.

In conclusion, we have demonstrated that Unisooth ST-32, by stimulating mechanisms linked to skin protection and repair, can indeed act as a long term soothing agent. Moreover its effect in reducing the TEWL can also suggest a role as a healing agent in restoring the damaged skin barrier. Unisooth ST-32 can be incorporated in products for daily protection and repair to provide a long term soothing and decrease the risk of environmental aggressions. Day and 24 hours products are certainly a suggested application, but Unisooth ST-32 can also be recommended in products for sensitive skin individuals where a constant soothing is needed. Recommended usage levels would be between 1.0% and 3.0%.

Unisooth ST-32 has been tested for skin tolerance, mutagenicity and biodegradability, and has provided an excellent safety profile.

**References**


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