A melanin booster for healthy skin

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For most people, a sun tan is a synonym of health and beauty. It has not been always like that however. Since the ancient times the idea of human beauty was a snow-white complexion. Only in the 1920s did people start perceiving sun tan in another way. However, it quickly became clear that an excessive exposure to the sun results in irreversible effects. UltraViolet Radiation (UVR) is a major environmental risk factor that contributes to carcinogenesis through DNA damage and immune modulation via inflammatory and immunosuppressive pathways.[1]

Darkened skin colour, the result of increased and redistributed epidermal melanin, is a familiar and well-studied response of normal skin to ultraviolet irradiation in humans. This tanning response has been shown to have two distinct phases termed immediate pigment darkening and delayed tanning.[2] Both components have strong genetic determinants and are generally far more pronounced in individuals with dark baseline (constitutive) pigmentation. Like all photobiologic responses, tanning requires direct interaction of UV photons with molecular targets in the skin. UVR directly affects epidermal melanocytes, the neural crest-derived skin cells responsible for melanin pigment production, skin pigmentation being considered as a natural protection against environmental insults. UV-induced melanogenesis also involves other cell types, among which keratinocytes appear to play the predominant role, secreting paracrine factors that enhance melanocyte survival, proliferation, dendricity and melanin synthesis. Keratinocytes also accept transferred melanin from melanocytes and thus the pigment is distributed more widely within the epidermis, increasing its photoprotective.[3]

Tyrosinase, a glycoprotein localised to the melanosome, is the principal and rate-limiting enzyme in melanin synthesis by virtue of its ability to catalyse tyrosine hydroxylation, the first reaction in the biosynthetic sequence. Several other enzymes are known to participate in melanin biosynthesis. These include tyrosinase-related proteins 1 and 2 (TRP1 and TRP2) and melanogenic inhibitors.[4]

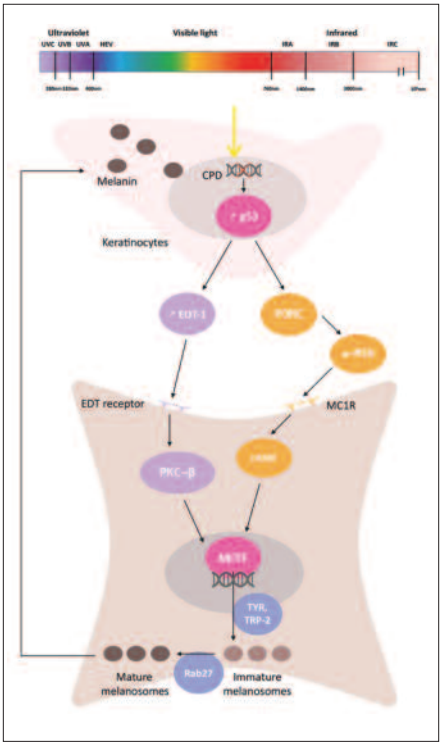

Figure 1: Tanning response to UV radiation. UV radiation induces DNA damage, which leads to activation of p53. In turn, p53 stimulates transcriptional upregulation of the proopiomelanocortin (POMC) gene, which is posttranslationally processed to α-melanocyte-stimulating hormone (α-MSH. Secreted α-MSH binds to the melanocortin 1 receptor (MC1R) on melanocytes, leading to production of melanin. The melanin is packaged within melanosomes and transported back to keratinocytes, where they localize over the nucleus as part of the protective tanning response to UV radiation.

The core component of the skin response to UV is the epidermal melanin unit, comprised of the melanocyte and its associated keratinocytes. UV exposure induces DNA damage in keratinocytes and results in stabilisation of the p53 tumour suppressor protein. This promotes p53 transcriptional activation of proopiomelanocortin (POMC), which is enzymatically cleaved to produce α–melanocyte-stimulating hormone (α-MSH). α-MSH is released by keratinocytes and binds the MC1R on melanocytes. MC1R activation by α-MSH triggers an increase in cAMP levels within the melanocytes, which increase transcription of microphthalmia-associated transcription factor (MITF) via CRE-binding protein/activating transcription factor 1. Binding of MITF to the E-box sequences in promoter regions triggers transcription of numerous pigmentation genes.[5] These genes act to synthesise, mature, and traffic melanin, the most common types of which are brown-black eumelanin and yellow-red pheomelanin. The melanin is packaged in melanosomes which are exported to keratinocytes (Fig 1).[6]

The tanning response relies heavily on UV-stimulated also increased production and release of numerous keratinocyte-derived factors including bFGF, NGF, endothelin-1 and beta-endorphin. These factors variably induce melanocyte mitosis, increase melanogenesis, enhance dendricity and prevent apoptotic cell death following the UV injury. Thus, events within the epidermal melanin unit conspire to maintain or increase melanocyte number, increase melanin production per cell, and enhance transfer of melanin pigment throughout the epidermis. Overall, UV-induced melanogenesis may be one part of a eukaryotic SOS response to damaging UV irradiation that has evolved over time to provide a protective tan in skin at risk of further injury from sun exposure.

In self-tanning (sunless tanning) products the substance dihydroxyacetone (DHA) is often applied as a standard ingredient. It

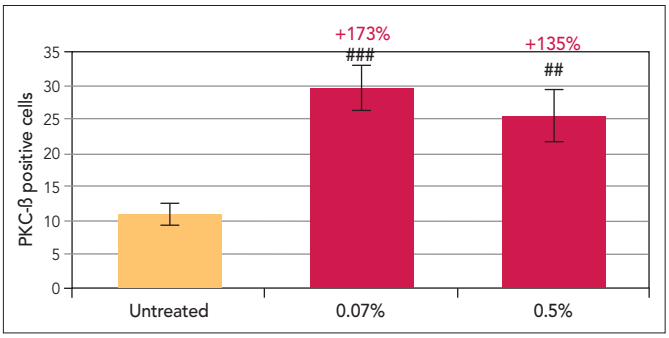

Figure 2. Effect of Epsiline® (red) on PKC-β production after 48 hours of treatment. ## p<0.01, ### p<0.001 vs not irradiated and untreated cells.

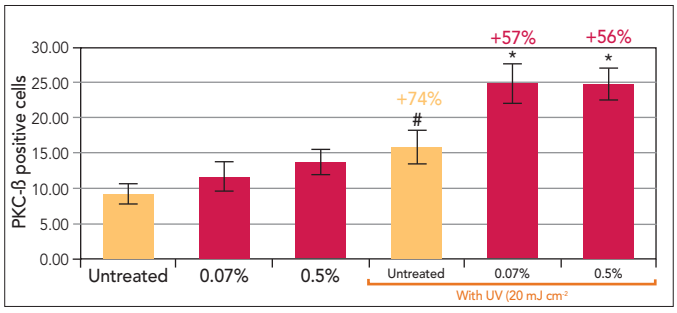

Figure 3. Effect of Epsiline® (red) on PKC-β production after 72 hours of treatment. # p<0.05 vs not irradiated and untreated cells, * p<0.05 vs irradiated and untreated cells.

binds to proteins of the stratum corneum and stains the upper layer of skin cells. The tan results from the so-called Maillard reaction between aldehydes or ketones and the amino acid lysine, leading to a coloured product. Unfortunately, such pigments do not absorb UV radiation and consequently do not provide any sun protection. In addition, the tanned outer cells peel off in the natural regeneration cycle and the skin turns pale again after a short time.[7]

It appears that molecules such as alginates, polysaccharides, derived from algae may have potential benefits on the mechanism described above. Among them, *Porphyridium cruentum* is considered to be one of the secrets to younger looking, besides benefits including anti-inflammatory activity, moisture, and UV protection.[8] Indeed, it is able to excrete complex matrices made of particular sugars (exopolysaccharides) with protective properties to survive. This microalga has been found to be a source of fatty acids (9-14%), proteins (28-39%) and polysaccharides (40-57%). Relying on the data of the literature, Greentech Group's goal was to provide a tanning activator and extender based on main regulator process of melanogenesis. Based on its knowledge of skin physiology and the marine world, Greentech Research developed a tanning activator active, named Epsiline®. With an average molecular weight of 130kDa, Epsiline® is composed of xylose, galactose, glucose and glucuronic acid and is charactersed by its high content of sulfated groups (21%)

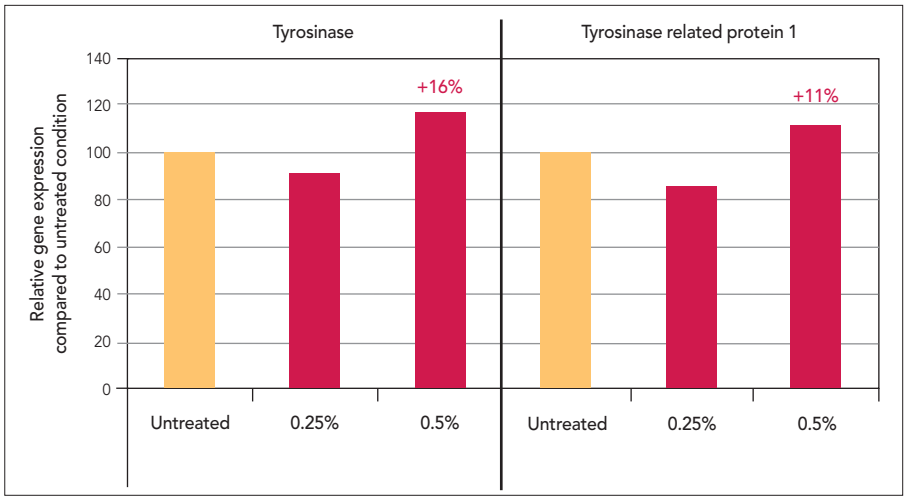

Figure 4. Effect of Epsiline® on gene expression after 48 hours of treatment.

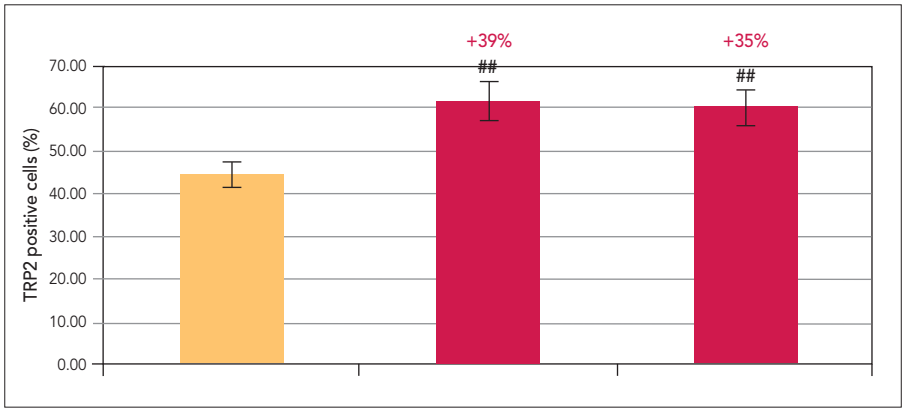

Figure 5. Effect of Epsiline® (red) on TRP-2 production after 48hours of treatment. ** p<0.01 vs untreated cells.

Results and discussion

Activation of melanogenesis process

We first evaluated Epsiline® (now referred to as 'the tanning active') on melanogenesis stimulation using a model of keratinocytes and melanocytes co-culture. The cells were cultured for 1 week before starting the treatments. All the cells plated were then treated 48 or 72 hours with the tanning active. All treatments were refreshed every day. 24 hours after the end of treatment, cells were irradiated (or not) at 20mJ.cm^{-2} twice. The markers were then analysed by immunocytochemistry and the results were quantified by image analysis.

The tanning active makes it possible firstly to activate PKC-β (a protein which regulates melanogenesis) without UVB radiation at 48h (Fig 2), and after 72h of treatment this activation is maintained by UV exposure (Fig 3). This active agent thus activates the melanogenesis in response to UVB by activating tyrosinase.

Activation of melanin synthesis

Using a culture of melanocytes, we highlighted that the tanning active induces Tyrosinase and TRP-1 gene expression after 48 hours of treatment without UVB. The genes expression was determined by RT-qPCR.

Using the model of keratinocytes and melanocytes co-culture. We noted that the tanning active activates TRP-2, one of the enzymes involved in the production of melanin from tyrosine, without UVB treatment. It is important to note that the tanning active maintains this activation in the presence or absence of UVB radiation after 72 hours of treatment (Fig 5).

Improvement of transfer to keratinocytes

To colour the skin, the mature melanocyte will transfer its melanosomes to surrounding keratinocytes. In order to deliver

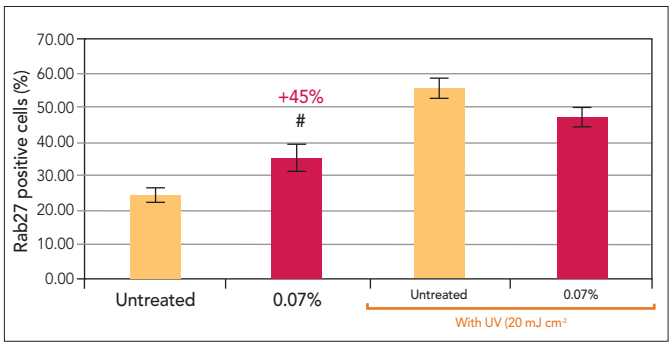

Figure 6. Effect of Epsiline® (red) on Rab27 production after 48 hours of treatment. # p<0.05 vs untreated and not irradiated cells.

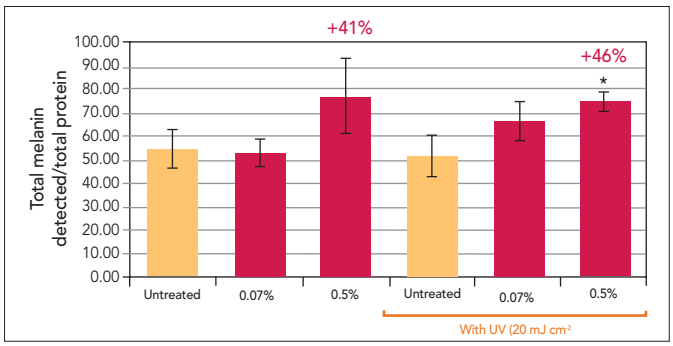

Figure 7. Effect of Epsiline® (red) on melanin present in the supernatant after 72 hours of treatment. * p<0.05 vs irradiated and untreated cells.

melanosomes to all surrounding keratinocytes, the melanocyte will develop dendrites. We studied melanocyte dendricity using the same keratinocytes-melanocytes model by labelling Rab27, which is a marker of melanosome transfer and differentiation. A 48 hours' treatment with the tanning active on non-irradiated cells induces a significant increasing number of Rab27 positive cells (+45%). When cells are irradiated, Rab27 expression is higher compared to non-irradiated cells (Fig 6).

Increasing synthesis and transfer of melanin with or without UVB exposure, allowing skin pigmentation

The determination of melanin in the supernatant corresponds to the end point of synthesis and transport of melanosomes to keratinocytes. The treatment with the tanning active, with or without UVB induction, induces significant increase in the release of melanin by melanosomes into the supernatant, with a dependent dose effect (Fig 7).

Activating and extending tanning

The beneficial effect of the tanning active versus placebo was evaluated using a Spectrophotometer®. It was an open, intra-individual study among 11 subjects from 18 to 40 years old during 14 days. After the determination of MED, the volunteers twice daily apply in their back a formula with the tanning active versus a placebo and were irradiated at Day 0, Day 3, Day 5 and Day 7.

Results showed that the tanning active presents a tan enhancing effect characterised by a significant decrease of L* and ITA°. The skin is darker and the pigmentation is reinforced. Moreover the tanning active shows a significant extender effect of tanning by maintaining it even 7 days after the last irradiation (Figs 8 and 9). This improvement was observed for 91% of the subjects for ITA° and 82% for L*.

Using a macrograph of the zone, we noted a darker skin for 64% of the subjects at Day 7. At D14, this visual effect was noted by 73% of the subjects.

Conclusion

Epsiline® is a melanin booster and has a tanning extender effect, through its stimulation of tyrosinase and melanin synthesis with and without UV exposure. Epsiline provides a healthy tan and additional protection against UV radiation, the principal cause of skin ageing. Epsiline thus ensures a sun-kissed complexion throughout the summer and makes it last, protecting the skin from deleterious effects of UV rays. PC

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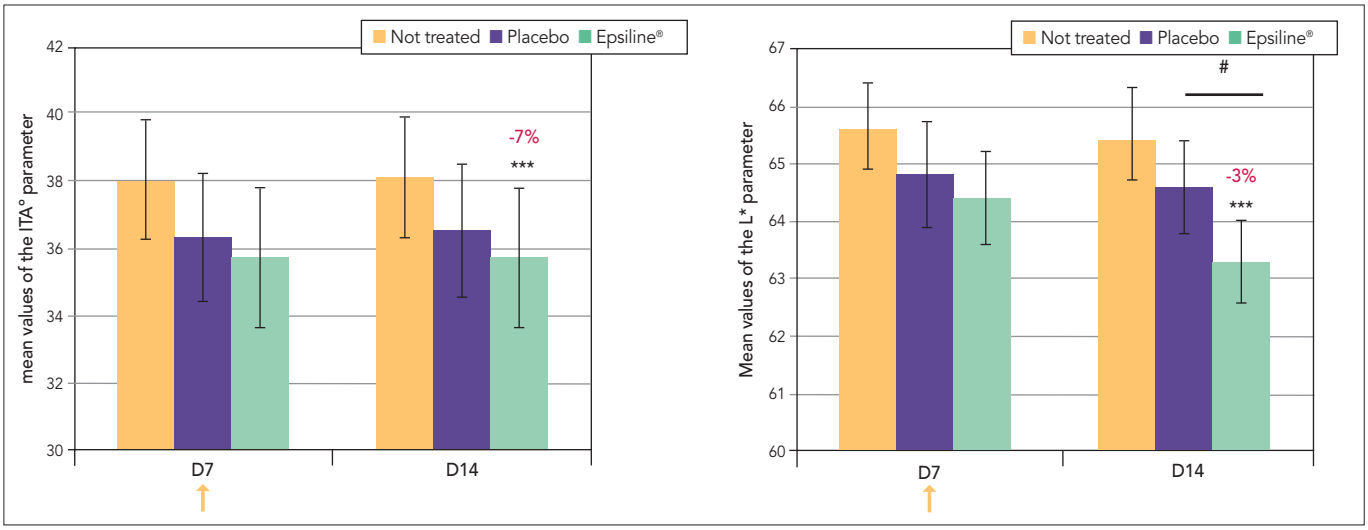

Figures 8 and 9. Effect of EPSILINE® on ITA° and L* parameters 7 days after the last irradiation. *** p<0.001 vs not treated area.