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The molecular mechanism of PCE-DP, a novel brightening active ingredient

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PCE-DP (D-pantothenyl alcohol) is an active ingredient in pharmaceuticals and cosmetics that prevents inflammation and promotes hair growth. In our previous randomized double-blind placebo-controlled UVB irradiation-induced pigmentation study using healthy Japanese men and women, we found that topical PCE-DP has brightening effects when used for 4 weeks. In this study, we examined four studies to clarify the molecular mechanism of PCE-DP activity: (i) epidermal turnover, (ii) melanosomal uptake of keratinocytes, (iii) melanocyte activation by keratinocytes, or (iv) melanin production in melanocytes. When PCE-DP was added to normal human epidermal keratinocytes (NHEK), the intracellular CoA and ATP concentrations significantly increased, and cell growth was significantly induced. Furthermore, epidermal turnover was enhanced by PCE-DP because the average projected area of corneocytes (APAc) significantly decreased after 4 weeks of daily topical application of PCE-DP to the skin of the medial upper arm. The mRNA expression level of adrenomedullin, a key factor for dendrite elongation in melanocytes, significantly decreased, and melanin uptake was inhibited by adding PCE-DP to the NHEK. The induction of endothelin-1 mRNA, which increases melanin synthesis in melanocytes, was significantly decreased by the addition of PCE-DP after UVB irradiation to the NHEK. On the other hand, when PCE-DP was added to melanocytes, no effects on cell growth or melanin synthesis were observed. This suggested that PCE-DP improves skin pigmentation by inducing epidermal turnover through the activation of ATP production in NHEK. This improvement mechanism may involve activation of the tricarboxylic acid cycle by increasing the intracellular CoA level. In addition, PCE-DP prevents skin pigmentation by inhibiting melanin uptake and inflammation after UVB irradiation in NHEK.

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Time-dependence of UVB induced cellular mechanisms in cultured human keratinocytes

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UVB-induced cyclobutane pyrimidine dimers (CPDs) are considered to be the main cause of acute sunburn and epidermal carcinogenesis. In humans, these lesions are repaired by nucleotide excision repair, but marsupials and lower organisms present photolyase enzyme which rapidly removes CPDs in a visible light-dependent process (photoreactivation). Previously we established an *in vitro* pseudouridin-modified mRNA encoding CPD-specific photolyase transfection on human keratinocyte cell lines, which was proved to be a proper method to avoid UVB-induced apoptosis. According to clinical experiences, there is a need for a treatment to diminish the deleterious effects of sunburn when UVB injury has already occurred and the first symptoms appear. Our aim was to determine the time interval after UVB exposure when keratinocyte apoptosis is still preventable. Normal human epidermal keratinocytes were transfected with lipofectamine-complexed, *in vitro* transcribed mRNA encoding CPD-specific photolyase. Cells were irradiated with 60 mJ/cm^2 UVB. At 0, 6, 8 or 12 hours after UVB cells were either exposed to visible light (photoreactivation) or kept in the dark. Viability was measured by Annexin V and propidium iodide dual staining followed by flow cytometry, the relative amount of intracellular CPDs was analyzed by CPD-specific ELISA. Photolyase-mediated CPD removal restored cell viability close to the baseline conditions 0 to 6 hours after UVB treatment. The effect of CPD removal on keratinocyte survival began to decrease 8 hours after the UVB damage. In mRNA-transfected and photoreactivated cells 80% of CPDs were removed at 6 hours post-irradiation. 8 to 12 hours after UVB 40-60% of the CPDs were eliminated. Our results suggest that UV-induced keratinocyte apoptosis can be prevented within 6-8 hours after the UVB injury by the elimination of CPD photolesions. After that point the effect of CPD removal on cell viability is less feasible.

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Enhanced UVA-induced cyclobutane pyrimidine dimer formation by silymarin without increased mutagenesis in cultured epithelial cells

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Silymarin is a phytophenol extracted from the seeds of milk thistle (Silibum marianum). Silymarin has been studied in skin UV-protection due to its antioxidant and anti-inflammatory effects. However, its possible phototoxic potential was also shown. Based on these findings the dermatological application of this polyphenol is questionable. Our aim was to study the effects of silymarin on cell viability, ROS production and mutagenesis in UVA-irradiated epithelial cells. HaCaT immortalized keratinocyte and CHO (Chinese hamster ovary) cell lines were treated with silymarin for 30 min, then exposed to 10 or 20 J/cm^2 UVA. Viability, ROS production, cyclobutane pyrimidine dimer (CPD) formation and HPRT gene mutagenesis of the cells were measured by flow cytometry, CPD-specific ELISA and HPRT gene mutation assay, respectively. In our experiments the antioxidant effect of the silymarin was demonstrated, but UVA-induced apoptosis was enhanced by silymarin treatment and silymarin showed photosensitizer properties. Silymarin-treated cells showed increased UVA-induced CPDs compared to controls. However, the higher CPD levels did not increase mutagenesis in the HPRT gene mutagenesis assay. Our results show that antioxidant and photoprotective effects of chemicals may be dyssynchronous and highlight the complexity of UV-mutagenesis. We also provide evidence for the complex effects of silymarin in modifying UV response and thus the need for careful consideration before using silymarin in dermatological preparations.

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Incisional biopsy-induced spontaneous regression with halo phenomenon in a congenital melanocytic nevus

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Congenital melanocytic nevus (CMN) is a hamartoma derived from neural crest appearing at birth. CMN has a dynamic course and may show various changes even spontaneous regression. CMN may grow in size during childhood and show pigmentary regression with increasing age and develop a hypopigmentation halo and regress spontaneously after halo formation, or undergo malignant transformation resulting in melanoma. A 9-year-old boy presented with solitary brownish to blackish patch on the right forearm which had appeared since birth. We performed incisional biopsy from the brownish patch and nests of nevus cells were observed in the entire dermis. The infiltration of melanocytes in adnexal-centric fashion was also shown. From these findings, the patient was diagnosed as CMN. Staging operations were additionally performed 5 times more to remove the lesion. After the first incisional biopsy, the brownish patch showed spontaneous regression with a halo phenomenon, especially around the suture sites. From the histologic findings, the regressed skin lesion showed perivascular cellular infiltration composed of lymphohistiocytes and decreased nevus cells. After the sixth incisional biopsy, the patch was completely removed, remaining halo around the suture sites. Spontaneous regression with a halo is a rare phenomenon in CMN. The mechanism is suggested to be a destruction of melanocytes by immune responses of CD 8+ T cells or IgM autoantibodies. There are some triggering factors that induce the process of halo phenomenon including UV radiation or local trauma. Also, inflammatory reactions associated with surgery may evoke the proliferation of CD8+ T cells targeting melanocyte. Herein, we report an interesting case in which surgical procedures act as a triggering factor to CMN regression with a halo phenomenon.

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Melasolv, a skin lightening compound with different mechanisms to regulate skin pigmentation

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The pigmentation of human skin (nevi, senile lentigines, melasma, etc) is regulated by a complex process involving the synthesis and distribution of melanin. Overexpression of melanin is induced by the off-balance between the signals which regulates melanin synthesis, this can be caused due to a response to external or internal stimulus that often affects the genes related to melanogenesis. To date, many studies have focused on developing direct enzymatic inhibitors of tyrosinase in order to achieve a skin-lightening effect. However, it was also reported that these direct inhibitors could be converted to quinone derivatives by tyrosinase-catalyzed oxidation, which lead to undesirable outcomes. Therefore, we have studied intrinsic inhibitory factors of melanogenesis in skin with the objective to achieve a lightening effect without the undesirable potential side effects. It was reported that non-coding RNAs including H19, miR125b and WIF1 were decreased in hyper-pigmented skin such as melasma and nevi. And it was found out that melanogenesis could be inhibited by increasing these lightening genes expression in normal human epidermal melanocytes and keratinocytes. Our group showed that the 3, 4, 5-trimethoxy cinnamate thymol ester (TCTE, Melasolv®), synthesized from gallic acid, is a lightening active which decreases tyrosinase protein formation by regulating the MITF. Likewise, we have also observed that Melasolv significantly decreased melanogenesis by increasing expression of the lightening genes H19, miR125b and WIF1 in the skin cells. Finally, we have confirmed the lightening effect of Melasolv in 3D human skin equivalent. Nonetheless, since Melasolv is not a direct inhibitor of tyrosinase enzymatic activity, it is extremely unlikely that nocive quinone derivatives conversions occur. From these results, we have proposed Melasolv as an active for lightening effects with a safer profile, when considering undesirable effects, to treat pigmentary disorder and uneven skin color.

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The role of photo-induced collagen degeneration in the development of telangiectasias in rosacea

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Disruption of the dermal matrix secondary to photoaging is one of the proposed etiologies for rosacea. Matrix metalloproteinase-1-mediated collagen degeneration has been shown to impact endothelial cells, leading to the formation of vascular tubes resembling telangiectatic lumina. We performed a case-control study to examine the relationship between clinical presentation, collagen degeneration, and microvascular changes between 5 patients with erythematotelangiectatic rosacea ages 47-83 and 5 controls matched by age ±5 years and race. Biopsies were taken of telangiectasias from rosacea patients and of normal facial skin from controls. Samples were stained with Picrosirius red for collagen visualization and CD31 for vessels. Image J and SPSS-25 were used for image analysis. When examining the papillary and reticular dermis of Picrosirius red-stained slides, samples from controls displayed significantly greater mean collagen content (19.603% ±8.821%) compared to rosacea patients (16.812% ±7.787%, p=0.030). Examination of the CD31-stained sections demonstrated a significantly higher mean microvessel density in rosacea patients (4.775 E-5 ±1.493 E-5 μm^{-3}) compared to controls (2.559 E-5 ±8.732 E-6 μm^{-3}, p=0.004) and significantly higher mean vessel lumen area in rosacea (491.710 ±610.188 μm^2) compared to controls (347.879 ±539.624 μm^2, p=0.003). By individual patient-control pair, 5/5 pairs had increased microvessel density and decreased collagen content in rosacea patients compared to controls. 3/5 pairs demonstrated significantly increased mean lumen area in rosacea individuals. No significant correlations were identified between the histologic findings and clinical severity grading of rosacea or photoaging. However, we identified a direct relationship between decreased collagen and increased microvessel size and density in rosacea patients. These structural changes may represent early aberrations that develop in this condition and provide a link between photodamage and rosacea.