Protective effect of Ethylhexyl Methoxycinnamate and Phytoene & Phytofluene against UV irradiation induced hair carbonylation

Kyung, Seoyeon[1]; Chae Da Eun[2], Kang, Seunghyun[1]
[1]R&I Center, COSMAX BTI, Seongnam 13486, Republic of Korea;
[2]R&I Center, COSMAX, Seongnam 13486, Republic of Korea;

Introduction:

Human are mostly exposed exclusively to solar ultraviolet (UV) radiation, which contains UVA and UVB that can be harmful to skin and hair. UVA and UVB have different properties and induce various reactions on the skin and hair, and UVB radiation is much more energetic than UVA radiation [1]. There is an increasing need for analysis of molecular mechanisms and biological events induced by UVA and UVB, which will be used as a method of clinical treatment and cosmetic application.

A number of reports indicate that UV radiation significantly disrupt the redox balance in human skin cell by overproducing reactive oxygen species (ROS) [2-4].Furthermore, ROS derived oxidative stress can be accumulated in macromolecules, which result in oxidatively damaged (carbonylated) proteins and lipids. Oxidative modified protein mostly shows reduced enzymatic activity, reduced stability to heat, increased hydrophobicity, and increased susceptibility to proteolysis degradation [5]. Also, oxidized lipids play an important role in the pathogenesis of oxidative stress-related human disorders such as atherosclerosis, obesity, inflammation and autoimmune diseases. And it has been reported that carbonylated glycerophospholipids can initiate and mediate chronic inflammation [6].

Human hair is mostly made up of keratin, which forms 65-95% of hair with insoluble cystine-containing helix protein complex [7]. Also, Hair lipids have been described as fatty substances loosely attached to the hair surface and can be easily extracted by lipid solvent [8]. Those hair lipids constitute mainly with sebum, oily secretions from the sebaceous glands of the scalp, and with lipids excreted as a by-product of epidermal keratinization [9]. Since most of the constituent materials of the hair surface are proteins and lipids, it was decided to measure carbonylated macromolecules as methods to study that can measure changes in the hair surface by UV.

Octylmethoxycinnamate (OMC, also known by ethyl-hexylmethoxycinnamate or octinoxate) is one of the most common sunscreens on the market in cosmetics to resist UVB from sunlight due to its excellent UV absorption curve, high lipophilicity and good oil solubility [10, 11]. By the way, the photo-instability of UV-B filters is an urgent issue, and therefore, they have received special attention today [12]. Also, OMC is listed as an endocrine disruptor compound (EDC) by the European Union's database [13] because of its potential risk for DNA damage associated with endocrine disrupting effects. In this sense, it was necessary to develop new formulations of sunscreens to stabilize or replace OMC and increase their protective effect, increasing their efficiency and safety for human health [14].

Phytoene (7,8,11,12,7ʹ,8ʹ-hexahydro-Ψ, Ψ-carotene, PT) and phytofluene (7,8,11,12,7ʹ,8ʹ-hexahydro-Ψ, Ψ-carotene, PTF) are special carotenoids as they are colorless, and precursors of all other carotenoids. Also, PT and PTF have light absorbing properties. PT absorbs maximally at 286nm, and PTF absorbs maximally at 348nm [15, 16]. As UVB radiations ranges from 290 to 320 nm, PT and PTF have possibilities to be used as sunscreens. Also, some studies suggest that PT and PTF can exhibit antioxidant capacity against 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation [17], which can be help structural stability when present together with structurally unstable substances. In addition, it has been reported that PT and PTF can protect against erythema and DNA damage induced by UV radiation and hydroxyl radicals, and may have anti-inflammatory effects as observed in human peripheral blood lymphocytes, in vitro, and mouse ear edema model, in vivo [18].

Based on the UV absorption ability of OMC, PT, and PTF, there have been quite a few studies on the skin protection effect, but the hair protection effect from UV has not yet been studied. Therefore, the purpose of our study is to visualize UV induced hair damage through staining of carbonylation, and to try to confirm the UV protective efficacies of OMC, PT and PTF.

Materials & Methods:

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Results & Discussion:

Protein carbonylation induced by both UVA and UVB

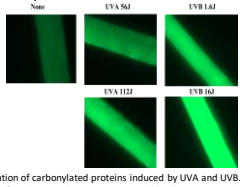

Figure 1. In situ visualization of carbonylated proteins induced by UVA and UVB. Carbonylated proteins were labeled in situ on hair shaft with CHH. They are represented by the green signal. Left panel : Image of a hair strand without UV irradiation. Middle panel : Image of hair shafts after UVA irradiation (56J, 112J). Right panel : Image of hair shafts after UVB irradiation(1.6J, 16J) Images were taken with a 40X zoom.

Results & Discussion:

Phospholipid carbonylation induced by both UVA and UVB

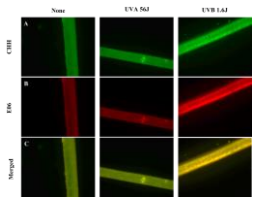

Figure 2. In situ visualization of carbonylated protein and lipids induced by UVA and UVB. Carbonylated proteins were labeled in situ on hair shaft with CHH. Carbonylated phospholipids were labeled in situ on hair shaft with E06. For better representation of co-staining, CHH stain shown in green(A), E06 stain shown in red(B). Merged images (yellow) demonstrate the co-localization of carbonylated phospholipid and carbonylated protein. Left panel: Image of a hair strand without UV irradiation. Middle panel: Image of hair shafts after UVA irradiation (56J). Right panel: Image of hair shafts after UVB irradiation(1.6J) Images were taken with a 40X zoom.

Protein and phospholipid carbonylation induced by UVB are prevented by OMC and UV-ene

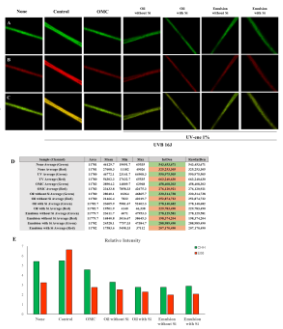

Figure 3. In situ visualization of carbonylated proteins and phospholipids induced by UVB in OMC or UV-ene treated hair shaft. Carbonylated proteins were labeled in situ on hair shaft with CHH. Carbonylated phospholipids were labeled in situ on hair shaft with E06. For better representation of co-staining, CHH stain shown in green(A), E06 stain shown in red(B). Merged images (C, yellow) demonstrate the co-localization of carbonylated phospholipid and carbonylated protein. Images were taken with a 20X zoom. The average fluorescence values from three different hair shafts are shown in the table for each channel (D), and the relative intensity is graphed based on this (E).

Effects of UV-ene on cell proliferation and hair growth factor expressions in DPC

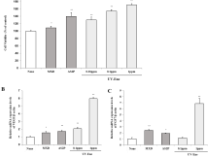

Figure 4. The effects of UV-ene on (a) cell proliferation and (b,c) mRNA expression levels of growth factors in DPCs. (a) DPCs were treated with indicated concentrations of UV-ene for 24 h. Cell proliferation was measured by the MTT assay. Results are expressed as mean values ± SE of three independent experiments. **p<0.01, *p <0.05 compared to the control. For mRNA expression levels, DPCs were treated with of UV-ene for 24 h. Relative mRNA expression levels of (b) VEGF and (c) FGF7 were measured by RT-qPCR. The means ± SEs are the average of three independent experiments. **p<0.01, *p <0.05 compared to the control.

Conclusions:

The present study demonstrates that UVA and UVB irradiation increase carbonylation of protein and lipid, an indicator of oxidative damage on the hair surface. In addition, by utilizing the principle of OMC, which is a UVB blocker, it suggests the possibility of expanding the use of formulations that can protect not only the skin but also the hair from UV rays. In addition, it was confirmed that UV-ene, a complex of PT and PTF, which are carotenoids similar to OMC, has a hair protection effect. By further confirming the HFDPC activating effects of UV-ene, UV-ene could be widely used in hair care formulations.

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Also, we would like to appreciate Matchfinder for advice on quantification of in situ study.

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