Poster ID – **P1036**

Attenuation of HEV blue light-induced fibroblast lipofuscin accumulation via a Triasorb-based formulation

Gautier Doat[1]*, Mauricio da Silva Baptista[2], Patrick Bogdanowicz[3], Carlos MV Palomino[2], Katherine Tsantarlis[2], Carine Jacques-Jamin[3], Camille Boudet[1], Hélène Duplan[3], Sandrine Bessou-Touya[3]

1. Medical Department, Laboratoires Dermatologiques Eau Thermale Avène, Pierre Fabre Dermo-Cosmetics & Personal Care, Lavaur, France

2. Department of Biochemistry, University of Sao Paulo, SP, Brazil

3. R&D Department, Pierre Fabre Dermo-Cosmetics & Personal Care, Toulouse, France

Introduction & Objectives

Chronic sun exposure is a major driver of skin damage, promoting oxidative stress, cellular aging, immunosuppression, hyperpigmentation and photocarcinogenesis. While sunscreens containing UVA and UVB filters provide extensive protection against erythema, photoaging and DNA damage, growing evidence indicates that visible light (400–700 nm) also contributes significantly to photoaging. Visible light accounts for approximately 45% of solar energy, penetrates deep into the dermis and generates over 50% of free radicals produced in human skin during sun exposure. High-energy visible light (HEVL), particularly violet (410 nm) and blue (450 nm) wavelengths, is the most photochemically damaging spectral region, associated with pigmentary disorders, hyperpigmentation and photoaging (Tonolli et al., 2023). Yet few sunscreen ingredients specifically target visible light-induced damage, highlighting the need for broader-spectrum photoprotection.

Lipofuscin, a fluorescent endogenous pigment composed of oxidized biomolecules, is a reliable biomarker of oxidative stress and cellular aging in multiple cell types, including keratinocytes and fibroblasts (Tonolli et al., 2017). Critically, lipofuscin itself acts as an endogenous photosensitizer: cells pre-loaded with lipofuscin exhibit heightened sensitivity to subsequent HEVL exposure, resulting in exacerbated ROS production and cytotoxicity, creating a self-amplifying cycle of oxidative damage that extends beyond the epidermis into the dermis. While this process is well characterized in keratinocytes, its impact on dermal fibroblasts remains poorly understood and largely underestimated.

A recently developed broad-spectrum filter, phenylene bis-diphenyltriazine (TriAsorb®), protects skin from UVA to visible light wavelengths. Previous studies demonstrated its efficacy against apoptosis and DNA damage induced by blue HEVL (Bacqueville et al., 2021). In the present study, we investigated whether HEVL induces lipofuscin accumulation and ROS production in fibroblasts and evaluated the protective effects of a TriAsorb®-containing sunscreen.

Materials & Methods

Fibroblasts (passages 4–11) were seeded in 24-well plates. After 48 hours of irradiation, lipofuscin accumulation was evaluated by intrinsic autofluorescence using confocal microscopy. Prior to imaging, cell nuclei were stained with DAPI to facilitate nuclear identification.

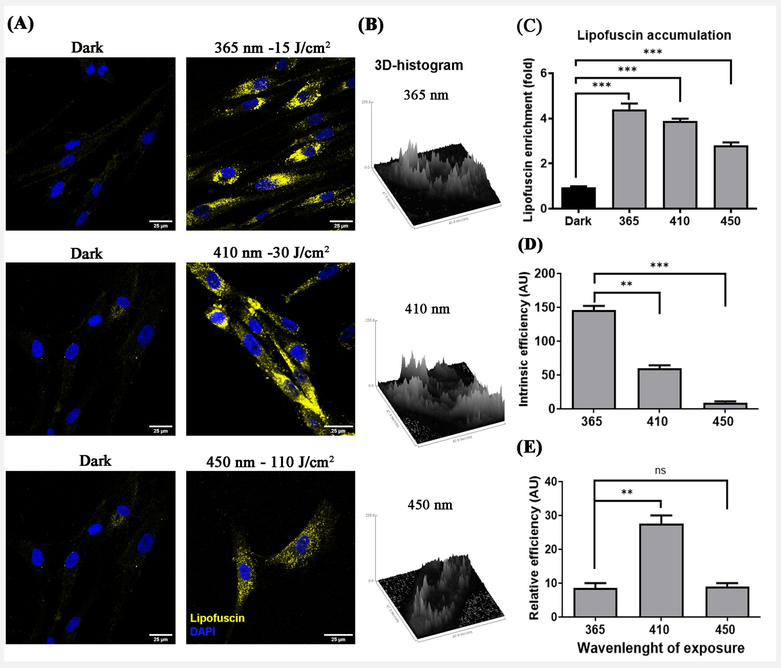
Intracellular ROS production was evaluated by oxidation of the fluorescent probe CM-H2DCFDA 48 h after irradiation

Formulation containing TriAsorb® were applied at 2 mg/cm² on top of the cell layers prior to light exposure.

Data are presented as mean ± SEM from independent experiments. Statistical significance was defined as ns ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$) relative to the indicated groups.

Results

Figure 1. Fibroblasts accumulate lipofuscin after UVA and VL exposure



(A) Representative images of primary fibroblasts exposed to 365, 410, and 450 nm light showing lipofuscin accumulation detected by intrinsic autofluorescence. Images were acquired using confocal microscopy (Leica SP8) with a 63× objective and 488 nm excitation. Scale bar: 25 µm. (B) 3D intensity histogram of the autofluorescence signal from a single-cell region of interest (ROI). (C) Quantification of autofluorescence intensity (%) in fibroblasts exposed to UVA, 410 nm, and 450 nm light, normalized from the dark group. Data are presented as mean ± SEM of independent experiments; analysis performed in ImageJ. (D) Relative efficiency of lipofuscin accumulation in skin exposed to light of 365, 410 and 450 nm. (E) Efficiency of lipofuscin accumulation in skin under solar exposure in real conditions.

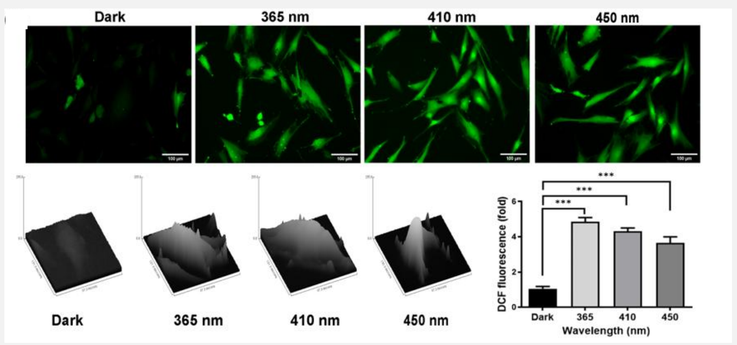
UVA, HEVL violet (410 nm) and HEVL blue (450 nm) induced significant lipofuscin accumulation in fibroblasts, as evidenced by increased autofluorescence (Figure 1A, B). The intrinsic efficiency of lipofuscin production decreased in the order: UVA > HEVL 410 nm > HEVL 450 nm (Figure 1C, D); however, when normalized to typical solar irradiance, HEVL 410 nm was estimated to be the major contributor to lipofuscin accumulation in fibroblasts under real-life sun exposure conditions (Figure 2).

Figure 2. Efficiency of lipofuscin accumulation in primary dermal fibroblasts upon UVA and HEVL exposure.

λ (nm)	Dose (J/cm²)	Lipofuscin fluorescence (a.u.) (a)	Photon energy (×10¹⁹ J)	Total photons delivered (×10¹⁸) (b)	Lipofuscin per photon (c)	Estimated relative contribution *in vivo* (Sun-normalized) (d)
356 (UVA)	15	378	5.6	2.7	140.0	7
410 (violet)	30	399	4.5	6.2	64.3	30
450 (blue)	110	190	4.4	24.9	7.6	8

a. Lipofuscin autofluorescence intensity (arbitrary units, a.u.) measured 48 h after irradiation.
b. Total photons delivered per cm², calculated as Dose (J/cm²) divided by photon energy (units of 10^18 photons/cm²).
c. Intrinsic photochemical efficiency: lipofuscin fluorescence per 10^18 photons (a.u. per 10^18 photons).
d. Estimated relative solar contribution: derived by multiplying (c) by the solar spectral photon flux (WHI 2008) integrated over a 10 nm band centered at each wavelength, normalizing so that the sum across the three wavelengths equals 100, and then scaling to convenient integers. This calculation accounts for both the efficiency of lipofuscin generation and the natural abundance of solar photons, indicating that 410 nm violet light is the predominant contributor under real world sun exposure.

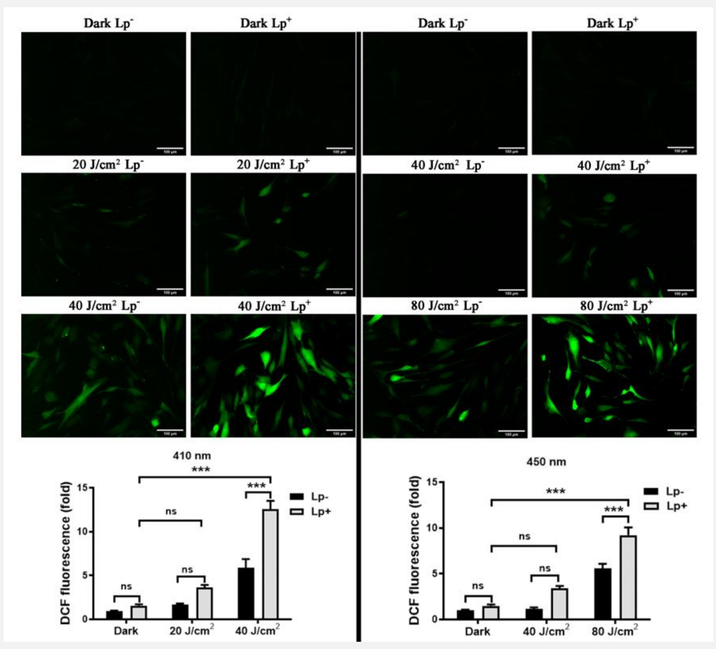
Figure 4. Effect of UVA and HEVL on ROS production



Representative fluorescence images of primary fibroblasts exposed to 365, 410, and 450 nm light showing ROS generation detected by CM-H_2DCFDA fluorescence. Images were acquired using a Leica DMi8 fluorescence microscope with a FITC filter and a 20× objective. Representative 3D intensity histograms of fluorescence signal distribution and quantification of ROS levels (%) normalized to the dark group (100%) are also shown. Data are shown as mean ± SEM from independent experiments and quantified using ImageJ. Statistical analysis was performed using one-way ANOVA followed by Tukey's test.

The effect of UVA and HEVL exposure and the redox balance of fibroblasts was evaluated by measuring ROS production, evident to increases in DCF fluorescence. All tested wavelengths resulted in a 3- to 4-fold increase in DCF fluorescence, following the same order in terms of the spectral variation, i.e., UVA > 410 > 450 nm.

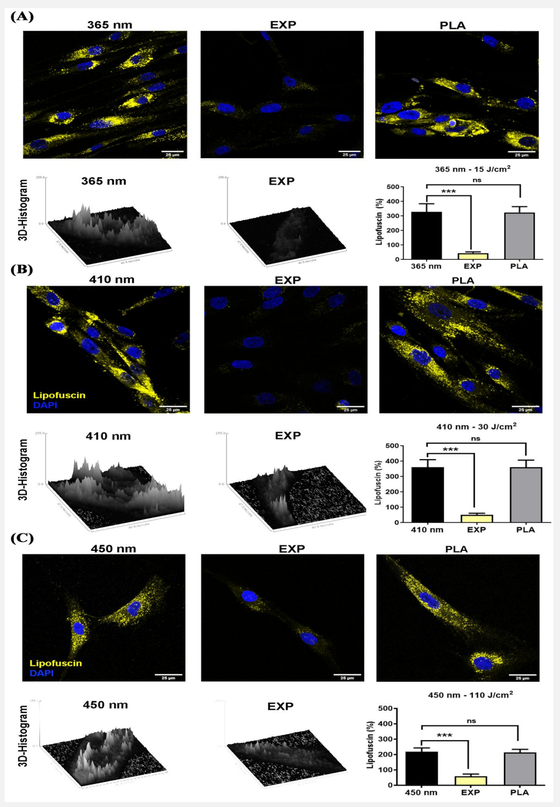
Figure 5. HEVL increases oxidative stress in lipofuscin-enriched fibroblasts.



Representative fluorescence images of primary fibroblasts exposed to 365, 410, and 450 nm light showing ROS generation detected by CM-H_2DCFDA fluorescence are shown. ROS quantification in fibroblasts with basal lipofuscin levels (Lp-) and lipofuscin-enriched fibroblasts (Lp+) following exposure to 410 nm (20 and 40 J/cm²) and 450 nm light (40 and 80 J/cm²) is presented together with representative fluorescence images. Data are shown as mean ± SEM from independent experiments and quantified using ImageJ. Statistical analysis was analyzed using two-way ANOVA followed by Tukey's test.

To assess the sensitizing effect of accumulated lipofuscin, cells were first pre-loaded by UVA irradiation (365 nm, 12.5 J/cm²) and subsequently exposed to either 410 nm or 450 nm HEVL. Lipofuscin-laden fibroblasts exhibited 2- to 3-fold higher ROS levels compared to non-pre-loaded controls, demonstrating that lipofuscin accumulation significantly amplifies HEVL-induced oxidative stress.

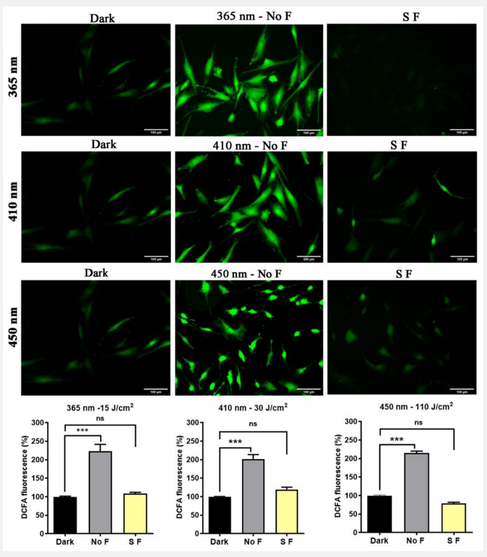
Figure 6. TriAsorb® reduces UVA- and HEVL-induced lipofuscin accumulation in fibroblasts.



Primary human fibroblasts were exposed to UVA (365 nm, 15 J/cm²), violet (410 nm, 30 J/cm²) or blue light (450 nm, 110 J/cm²) with or without sunscreen formulation (SF or No F). Lipofuscin-associated autofluorescence was assessed by confocal microscopy 48 h post-irradiation. Intense cytoplasmic autofluorescence was observed in No F groups across all irradiation conditions, whereas SF-treated fibroblasts exhibited markedly reduced autofluorescence signals, confirmed by 3D fluorescence histograms (A: UVA; B: 410 nm; C: 450 nm). Quantification normalized to dark controls demonstrated substantial reductions in lipofuscin accumulation in SF-treated cells compared to No F groups.

Application of the TriAsorb®-containing sunscreen formulation markedly reduced lipofuscin accumulation in fibroblasts exposed to UVA, 410 nm and 450 nm HEVL, achieving reductions of 99%, 97% and 89%, respectively, compared to unprotected irradiated cells. In contrast, the placebo formulation provided no significant protection, with lipofuscin levels remaining comparable to those observed in light-exposed, untreated cells.

Figure 7. TriAsorb® attenuates UVA- and HEVL-induced ROS production in fibroblasts



Primary human fibroblasts were exposed to 365 nm (15 J/cm²), 410 nm (30 J/cm²), or 450 nm light (110 J/cm²) in the presence of sunscreen formulation (SF) or without formulation (No F). Intracellular ROS production was evaluated 48 h after irradiation using CM-H_2DCFDA fluorescence. Representative fluorescence images and quantification of ROS levels (%) normalized to the dark group (100%) are shown. Statistical analysis was performed using one-way ANOVA followed by Tukey's test.

In unprotected cells, all tested wavelengths induced a 2- to 3-fold increase in ROS production compared to dark controls. Pre-application of the sunscreen formulation significantly reduced ROS levels to values comparable to those of dark controls. Notably, even in lipofuscin-laden fibroblasts, the sunscreen effectively prevented HEVL-induced ROS overproduction, restoring oxidative balance to levels similar to unexposed control cells.

Conclusions

- **Dermal fibroblasts accumulate lipofuscin upon UVA and HEVL exposure, extending HEVL-induced photoaging beyond the epidermis into the dermis.**
- **Violet light (410 nm) is the major contributor to lipofuscin accumulation under realistic solar irradiance conditions.**
- **Lipofuscin acts as an endogenous photosensitizer, amplifying HEVL-induced ROS production by 2- to 3-fold in pre-loaded fibroblasts — creating a self-reinforcing cycle of cumulative oxidative damage.**
- **The TriAsorb®-containing sunscreen effectively blocked this cascade, reducing lipofuscin accumulation by up to 99% and restoring ROS levels to those of unexposed controls, even in lipofuscin-laden cells.**
- **Broad-spectrum photoprotection extending into the visible light range is essential to prevent cumulative dermal photoaging and oxidative stress.**

Acknowledgement

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References

Tonolli et al. (2023). The Phototoxicity Action Spectra of Visible Light in HaCaT Keratinocytes. J Photochem Photobiol B, Jun:243:112703.

Tonolli et al. (2020). Lipofuscin in Keratinocytes: Production, Properties, and Consequences of the Photosensitization with Visible Light. Free Radic Biol Med 20:160:277-292.

Bacqueville et al. (2021) Phenylene bis-diphenyltriazine (TriAsorB), a new sunfilter protecting the skin against both UVB + UVA and blue light radiations. Photochem Photobiol Sci. 20:1475-1486.

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