Protection strategies to inhibit blue light irradiation effects *in-vitro* and in skin *ex-vivo*.

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Abstract/Introduction

The negative impacts of visible light, particularly blue light (wavelength 380-500nm), to skin are still not fully understood. A few studies have shown an increase of blue light-induced oxidative stress and hyperpigmentation suggesting a contribution to photo-aging (1-3). Only two UV-filters were found to partially block wavelength >380nm, hence additional means of protection are needed. We investigated blue light-induced cutaneous damages and screened for compounds to suppress these damages. Vitamins B₃, B₆ and E could prevent beta-carotene from blue light-dependent degradation *in-vitro* on PMMA plates. To investigate the potential of such compounds to suppress blue light induced damages in skin, we developed an *ex-vivo* model of blue light irradiation. Using fluencies of up to 100 Jcm⁻² we could show a dose-dependent increase of reactive oxygen species, and we established protein carbonylation as a novel marker of blue light induced skin damage. We found that distinct compounds like vitamin B₃ as well as an extract of the freshwater microalga *Scenedesmus rubescens* could suppress blue light induced damage significantly (p<0.05) *ex-vivo*.

Methodology

Beta-carotene test

The beta-carotene test (BCT) was performed as described previously (4). Under red light, we applied 2 µLcm⁻² of beta-carotene (0.5%(w/w)) dissolved in o-xylene to PMMA plates sandblasted to 2 µm roughness (Schönberg GmbH, Hamburg, Germany). We then dried the plates in the dark (RT, 10 min) and applied the test formulation homogeneously to the top of the plate (2 µLcm⁻²) using a finger. The plates were fixated in the Atlas SunTester XLS+ (Atlas Material Testing Technology GmbH, Linsengericht, Germany) and irradiated in the range of 290-800nm with 329 kJm⁻². In a second test, we applied UV light cut-off filters Schott GG400 (Schott AG, St. Gallen, Switzerland) on top of the fixation and irradiated again. These UV cut-off filters are dense for wavelengths below 400nm. The irradiation dose was 246 kJm⁻². Beta-carotene was dissolved in 50 mL isopropanol and put in an ultrasonic bath for one minute. Beta-carotene was photometrically quantified at 452nm. For each formulation, we prepared a minimum of three plates.

Analysis of blue light-induced reactive oxygen species *ex-vivo*

We acquired skin biopsies from abdominal plastic surgery, following Helsinki declaration and with the patient's informed consent, from Caucasian female donors aged 65. The detection probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was incorporated into the culture medium for 30 minutes before the irradiation, following the method described by Marionnet *et al* (5), which we adapted for blue light. Compounds were administered topically before and immediately after blue light irradiation (100 Jcm⁻², 380-470nm, max at 420nm, from a UV436 HF by Herbert Waldmann GmbH Co, Villingen-Schwenningen, Germany). Twenty-four hours after irradiation, the skin samples were harvested, cryo-fixed and cut for subsequent image acquisition and analysis. For each image the upper dermis was analyzed by evaluating the fluorescence through Image-J application (NIH, USA). The value obtained was then normalized against the dimension of the selected area.

Analysis of blue light-induced carbonylated proteins *ex-vivo*

We used abdominal plastic surgery skin biopsies from Caucasians female donors aged 65. Compounds were administered topically to skin tissue at the concentrations indicated in figure 6. After administering compounds before and immediately after blue light irradiation (100 Jcm⁻²) we extracted carbonylated proteins from the epidermal part of the skin tissues 48 hours after irradiation with blue light (380-470nm, max at 420nm, UV436 HF by Herbert Waldmann GmbH Co, Villingen-Schwenningen, Germany). After harvesting, the frozen skin samples were weighed and minced. Total cell lysates were then recovered by centrifugation. We determined carbonylated proteins by western blot and chemiluminescence detection, using an OxyBlot Protein Oxidation Detection Kit (Merck Millipore, Darmstadt, Germany, #S7150) according to the manufacturer's instructions. The range of the carbonyl content obtained for each sample was normalized against the actin protein signal band.

Conclusion

Our data provide further evidence that blue light can promote harmful effects like oxidative damage in skin. It acts by potentially decreasing the skin's own protective mechanisms, including beta-carotene content (6). Protection beyond the UV spectrum of solar irradiation should therefore become paramount in protecting skin against sun-induced aging. As a result of this study we propose a three-dimensional protection strategy for skin by using UV filters having a reduced transmission in the blue light spectrum, vitamins such as vitamin B₃, B₆, and vitamin E as well as biological actives like an extract of *Scenedesmus rubescens* to defend skin on different levels against solar irradiation.

References

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Results

Table 1:

INCI	A	B	C	D
Diisopropyl Sebacate	3	3	3	3
Dimethicone	2	2	2	2
Stearyl Alcohol	1.5	1.5	1.5	1.5
Potassium Cetyl Phosphate	2	2	2	2
Dicaprylyl Ether	2	2	2	2
Isopropyl Myristate	14	11	14	11
Titanium Dioxide; Silica; Dimethicone	/	3	/	3
Phenoxyethanol; Ethylhexylglycerin	1	1	1	1
Hydroxyethyl Acrylate/ Sodium Acryloyldimethyl Taurate Copolymer	0.5	0.5	0.5	0.5
Polyacrylate Crosspolymer-6	0.5	0.5	0.5	0.5
Silica	3	3	3	3
Aqua	65.5	65.5	57.5	57.5
Propanediol	5	5	5	5
Methylene Bis-Benzotriazolyl Tetramethylbutylphenol; Aqua; Decyl Glucoside; Propylene Glycol; Gellan Gum	/	/	8	8

Table 2:

INCI	E	F	G	H	I
Bis-Ethylhexyloxyphenyl Methoxyphenyl Triazine	1.5	1.5	1.5	1.5	1.5
Polysilicone-15	1	1	1	1	1
Butyl Methoxydibenzoylmethane	2	2	2	2	2
Ethylhexyl Salicylate	5	5	5	5	5
Octocrylene	1.5	1.5	1.5	1.5	1.5
Diisopropyl Sebacate	3	3	3	3	3
Dimethicone	2	2	2	2	2
Dicaprylyl Ether	2	2	2	2	2
Titanium Dioxide; Silica; Dimethicone	3	3	3	3	3
Potassium Cetyl Phosphate	1.5	1.5	1.5	1.5	1.5
Stearyl Alcohol	3.15	3.15	3.15	3.15	3.15
Hydroxyethyl Acrylate/ Sodium Acryloyldimethyl Taurate Copolymer	0.4	0.4	0.4	0.4	0.5
Polyacrylate Crosspolymer	0.4	0.4	0.4	0.4	0.5
Phenoxyethanol; Ethylhexylglycerin	1	1	1	1	1
Silica	3	3	3	3	3
Aqua	56.1	55.1	53.1	52.1	52.4
Propanediol	5	5	5	5	5
Tromethamine	0.45	0.45	0.45	0.45	/
Methylene Bis-Benzotriazolyl Tetramethylbutylphenol; Aqua; Decyl Glucoside; Propylene Glycol; Xanthan Gum	8	8	8	8	8
Pyridoxine Hydrochloride	/	1	/	1	/
Niacinamide	/	/	3	3	3
Tocopherol	/	/	/	/	0.5
Scenedesmus Rubescens Extract	/	/	/	/	1
Chlorphenesin	/	/	/	/	0.2
Parfum	/	/	/	/	0.2

Figure 1: Spectroscopic measurement of protection of beta-carotene after full spectrum irradiation. The PMMA plates, with the test formulations, were irradiated with the full spectrum (290-800nm). The grey bar corresponds to the non-irradiated beta-carotene. The achieved value was set as 100%protection of beta-carotene. The irradiated chassis formulation showed significant degradation of beta-carotene. The result of this formulation was set to 0%protection, and the results of all other formulations were calculated compared to that. A: chassis formulation (Table 1) with and without irradiation; B: with 3% titanium dioxide; C: with 4%MBBT (Methylene bis-benzotriazolyl tetramethylbutylphenol); D: with 3%titanium dioxide and 4%MBBT.

Figure 2: Spectroscopic measurement of protection of beta-carotene after full spectrum irradiation. The PMMA plates, with the test formulations, were irradiated with the full spectrum (290-800nm). The grey bar corresponds to the non-irradiated beta-carotene. The achieved value was set as 100%protection of beta-carotene. The irradiated chassis formulation showed significant degradation of beta-carotene. The result of this formulation was set to 0%protection and the results of all other formulations were calculated compared to that. E: chassis formulation (Table 3) containing UVB and UVA filters with and without irradiation. G: with 3% niacinamide (vitamin B₃); I: with 3%vitamin B₃ and 0.5%dl-alpha-tocopherol (vitamin E).

Figure 3: Spectroscopic measurement of protection of beta-carotene after irradiation with wavelengths ≥400nm. The PMMA plates, with the test formulations, were irradiated with visible light only (400-800nm). The grey bar corresponds to the non-irradiated beta-carotene. The achieved value was set as 100%protection of beta-carotene. The irradiated chassis formulation showed significant degradation of beta-carotene. The result of this formulation was set to 0%protection and the results of all other formulations were calculated in comparison. E: chassis formulation (see table 3) containing UVB and UVA filters with and without irradiation. F: with 1%pyridoxine hydrochloride (vitamin B₆); G: with 3%niacinamide (vitamin B₃); H: with 3% vitamin B₃ and 1%vitamin B₆; I: with 3%vitamin B₃ and 0.5%dl-alpha-tocopherol (vitamin E).

Figure 4: Inhibition of blue light induced dermal ROS formation. Vitamin B₃ and the *Scenedesmus* extract were able to inhibit ROS formation significantly. * p<0.05, ** p<0.01; *** p<0.001; all against blue light irradiated vehicle control by unpaired t-test.

Figure 5: Inhibition of blue light-induced epidermal protein carbonylation. Vitamin B₃ and the *Scenedesmus* extract were able to inhibit protein carbonylation significantly. Vitamin B₆ showed a tendency towards inhibition. * p<0.05; ** p<0.01; all against blue light irradiated vehicle control by unpaired t-test.