

Mycosporine-like Amino Acids from Red Algae Protect against Premature Skin-Aging

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Abstract: Skin ages in two ways: one is slow and normal and the other is the rapid aging induced by sunlight. UV-screening compounds to reduce damage caused by ultraviolet (UV) radiation are almost ubiquitous in nature. The most active natural UV-absorbing substances are the mycosporine-like amino acids (MAA) which are produced by certain algae, corals and zooplankton. The peak absorption of MAA is in the UVA range and their absorption coefficients are similar to those of synthetic sunscreens. Two *in vitro* studies demonstrated a DNA-protecting effect and the viability enhancing properties of MAA from the red alga *Porphyra umbilicalis*. A human study showed that a cream containing MAA efficiently protects the skin against UVA-induced lipid oxidation.

Introduction



Normal skin aging is accompanied by slow and continuous structural, functional, and metabolic changes in the skin. Such changes are greatly accelerated when the skin is exposed to solar UV radiation. The solar UV spectrum which reaches the earth's surface has been divided into UVB (290 - 320 nm) and UVA (320 - 400 nm). The more energetic UVB is absorbed in the epidermis where it can cause acute sunburn, DNA mutation or even cancer. Although less energetic, the longer wavelengths in the UVA range can penetrate much deeper into the skin. UVA reaches the dermis where it is responsible for the premature skin aging effects of sunlight.

UVA alters the expression of certain genes by generation of reactive oxygen species (ROS) and / or stimulation of pro-inflammatory cytokines such as IL-1 β and TNF- α . It has been shown that UVA activates the transcription factors NF- κ B and AP-1. This results in the induction of a series of collagen and elastin-degrading enzymes, the so called matrix metalloproteinases (MMP). A decrease in collagen content and fiber fragmentation ultimately leads to the typical signs of photoaging. ROS are formed after absorption of UV through skin chromophores, like urocanic acid or DNA. When UV light is absorbed by trans-urocanic acid (absorption maximum at 345 nm), singlet oxygen is generated. This highly reactive radical can attack proteins and lipids. The reaction products, such as lipid peroxides, are themselves ROS and have lost cellular functionality.

While UVB is highly dependent on the season, day-time, cloudiness, and latitude, UVA is relatively constant during the year. In central Europe, erythematous UVB is of minor importance for daily skin care

products, but UVA is present all year round in harmful doses and can penetrate indoors as well since UVA easily passes through window glass. To estimate individual daily UV exposure test persons were equipped with small UV counters by the research group of Rudolph and Träger¹. Exposure was measured on typical working days, taking into account also indoor UV radiation. Their data showed that indoors, UVB is, in contrast to UVA, irrelevant. A daily UVB dose of 2.5 MED in front of open windows on a sunny summer day is reduced to 0.2 MED when the windows are closed. But about 40% of the outdoor UVA radiation was still recorded indoors. It is therefore UVA-protection and not SPF factors that are of real relevance for daily skin care. We analyzed the potential of natural UVA-screening substances to protect the skin against photoaging.

The strongest UVA-absorbing compounds in nature are the mycosporine-like amino acids (MAA). They were found in different organisms such as cyanobacteria, red algae, dinoflagellates, corals, and many marine invertebrates. The basic cyclohexanone or cyclohexenimine chromophore is responsible for UV absorbance. Incorporation of various amino acids or iminoalcohol groups results in a diversity of about 20 MAA. The red alga *Porphyra umbilicalis* is reported to produce the MAA Porphyra-334 (Fig. 1) and Shinorine². Their absorption coefficients (ϵ_{molar}) at 334nm are 42'300 and 44'700, respectively. Their filter capacity is therefore similar to that of synthetic UVA sunscreens such as Parsol[®] 1789 (ϵ_{molar} 40'000) and Mexoryl[®] SX (ϵ_{molar} 45'000). In the food industry *Porphyra umbilicalis* is commonly known as nori, the most widely consumed seaweed in the world. In Japan nori is used to prepare sushi dishes.

This article summarizes study results on the efficacy of a cream containing MAA extracted from *Porphyra umbilicalis* in the prevention of photoaging induced by UVA irradiation as well as *in vitro*

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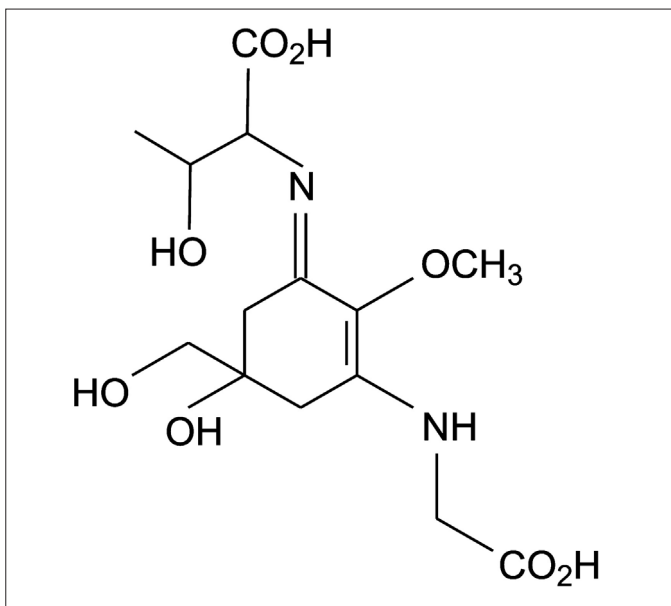


Figure 1: Mycosporine-like amino acid (MAA): *Porphyra 334*

results regarding the protection properties of MAA against UVA-caused DNA damage and loss of cell viability.

Materials and Methods

Extraction of MAA

3.3% dried *Porphyra umbilicalis* material was suspended in 15% ethanol and extracted after incubation at 45°C for 2 hours under constant stirring. After removal of the algae material the extract was clarified by ultra-filtration through a 10 kDa cut-off membrane. The test substance was prepared by mixing the clear extract with 3.3% lecithin in form of liposomes and 0.4% phenoxyethanol. The MAA concentration in the test substance (Helioguard 365) was adjusted to 0.1%.

In Vitro Studies

Protection of Cell Viability (MTT Test)

Keratinocytes (human HaCaT) were cultured in DMEM H.G. containing 10% fetal calf serum (FCS) in an incubator at 37°C with 5% carbon dioxide. Cells were incubated for 24 hours with different concentrations of Helioguard 365 (none, 0.125% and 0.25%). They were irradiated with UVA at 320nm for 10 minutes in 24 well plates, another series stayed untreated as control. After another 48 hour incubation in culture medium, the viability was measured applying the MTT test.

Protection against DNA Damage (Comet Assay)

Fibroblasts (IMR-90) were incubated in 0.1% FCS for 48 hours. After washing, the cells were covered with test samples (0%, 3%, and 5% of Helioguard 365). They were then irradiated under a UV-lamp with an emission maximum in the UVA range. After irradiation, the

cells were incubated for 30 minutes in culture medium. For the Comet assay, the cells were trypsinized and cell lysis was processed in low melting point agarose gel. After electrophoresis, the gels were stained with Sybr Green II and the tails were measured with a fluorescent-microscope. For each test at least 50 cells were measured and the average value was calculated.

Human Study

In a study over four weeks with 20 women in the age of 36 to 54 the following skin parameters were analyzed: elasticity by means of Cutometer SEM 575 (Courage & Khazaka GmbH; Cologne, Germany), roughness using the digital micromirror device PRIMOS (GF Messtechnik GmbH; Teltow, Germany), depth of wrinkles with the optical 3D in vivo measurement system PRIMOS and lipid peroxidation by analysis of squalenehydroperoxide formation (HPLC).

The test products were a base cream with 5% of Helioguard 365 (final MAA concentration of 0.005%) and the same base with 4% of a synthetic UVB sunscreen and 1% of a synthetic UVA sunscreen. The base cream without actives served as the control. The test products were applied twice daily to the inner side of the forearm and on the face (only the cream containing MAA to measure depth of wrinkles). The test areas on the forearm were irradiated twice weekly with UVA (10 J/cm²).

Results and Discussion

Studies on Stability

Stability tests, assessed by means of the content of mycosporines, revealed that the extract showed no reduction after one month at temperatures of 4°C, room temperature, and 37°C, respectively. After three months, the extract was still stable at 4°C and room temperature. At 37°C, the mycosporine content had decreased by 20%. Stability tests additionally applying UVA irradiation at the same three temperatures showed no loss of mycosporines content after three months (Fig. 2).

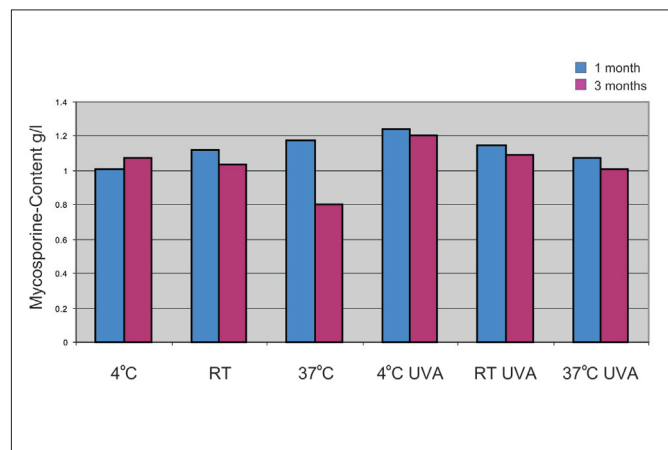


Figure 2: Stability of MAA's in Helioguard 365

In Vitro Efficacy Studies

Protection of Cell Viability

The result reveals that UVA irradiation impaired the viability of cells by 35.2%. Helioguard 365 in the concentrations of 0.125% and

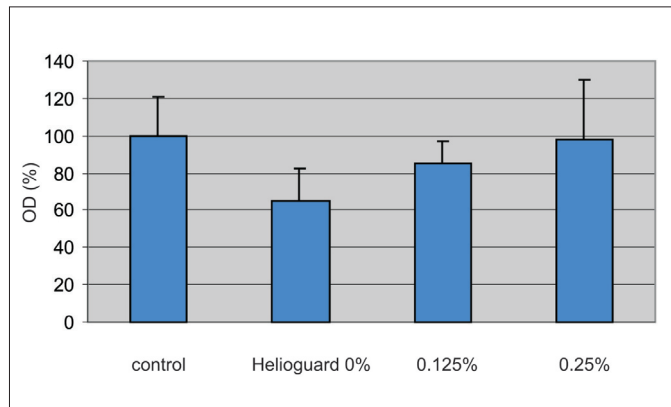


Figure 3: Viability of keratinocytes after UVA irradiation

0.25% improved the viability in a dose-dependent manner. The addition of 0.25% Helioguard 365 resulted in almost the same viability of irradiated cells as non-irradiated ones. Viability after irradiation and incubation with 0.25% Helioguard 365 amounted to 97.8%. Hence, Helioguard 365 is clearly able to protect the cells against UVA irradiation (Fig. 3).

Protection against DNA Damage

The length of the tails of the spots in the stained gels indicate the extent of DNA damage. Irradiation of the cells led to serious degradation of the DNA as shown in the gel after irradiation without Helioguard 365 treatment (Fig. 4). The addition of 3% and 5% of

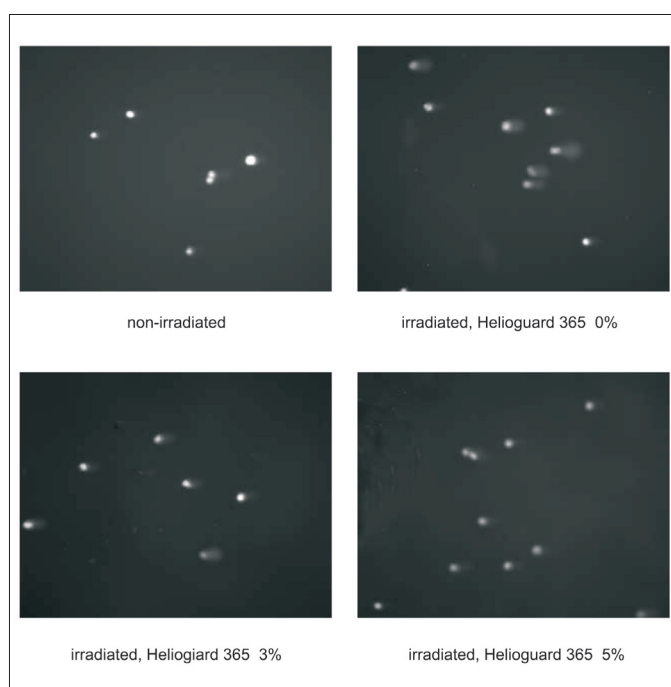


Figure 4: Comet Assay: The length of the tails indicates the extent of DNA damage

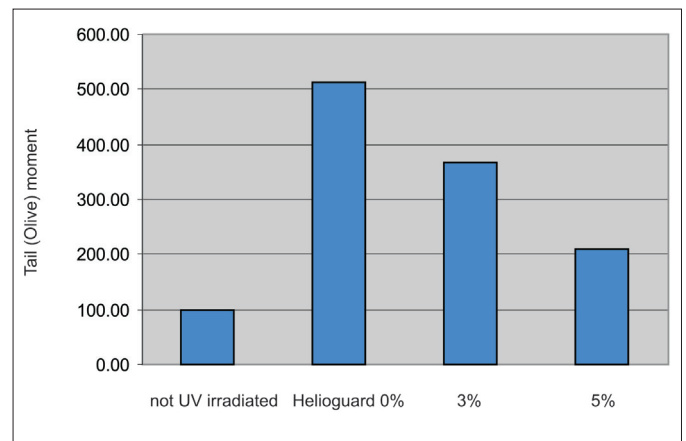


Figure 5: Tail (Olive) Moment: The bars represent the numerical values of the tails shown in figure 4

Helioguard 365 visibly diminished the damage to the DNA in a dose-dependent manner. Figure 5 shows the length of the tails numerically. This experiment demonstrated the excellent preventive efficacy of Helioguard 365 against DNA damage caused by UV irradiation.

Human Study

Skin is normally subjected to UVA exposure of between 3 and 20 J/cm². Rudolph and Träger measured UVA exposure of 8 J/cm² on the outside of the forearm held facing a window, during a cloudy and sunny day. The irradiation dose, which was applied in this study (two times 10 J/cm² per week), corresponds therefore to an average UVA exposure at a non-protected skin site. Thus, the study mimicked the daily photoaging of skin, and the protection provided by creams.

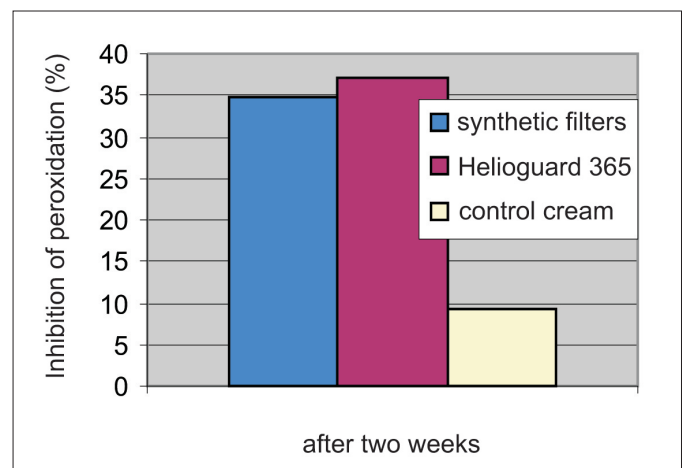


Figure 6: Inhibition of lipid peroxidation compared to the beginning of the study and the untreated area

The study results demonstrated that both test products could reduce the primary damage caused by UVA irradiation such as lipid peroxidation by about 35% (Fig. 6). Application of the standard cream without actives could minimally inhibit (10%) lipid peroxidation. After being applied for only 2 weeks, the test products significantly reduced the secondary effects of UVA exposure as shown by

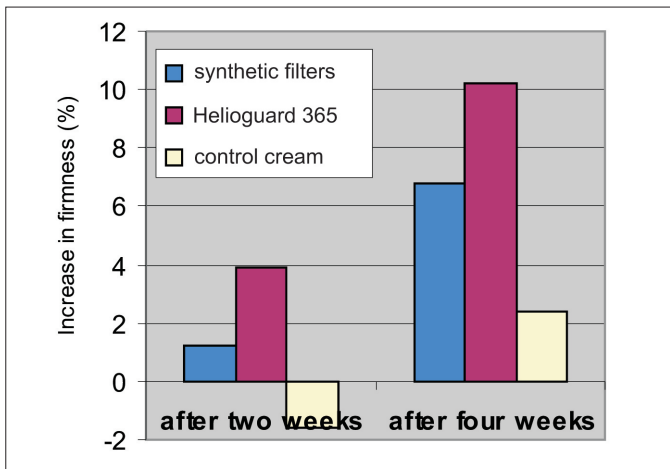


Figure 7: Improvement of skin firmness

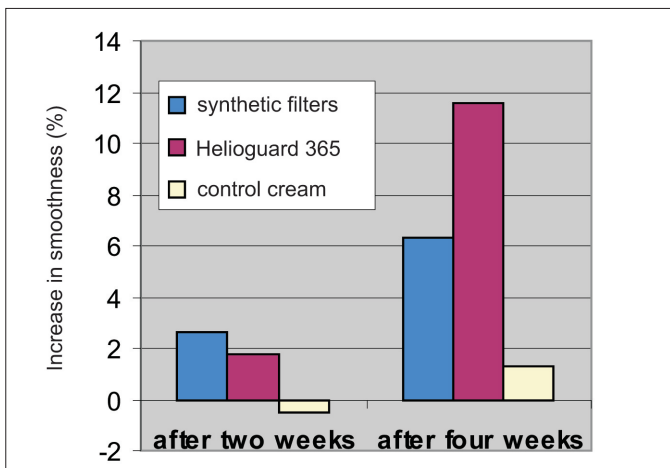


Figure 8: Improvement of skin smoothness

improved skin firmness and smoothness. After four weeks, these parameters were improved by more than 10% in areas treated with the Helioguard 365 test cream and by about 6% in zones treated with synthetic filters (Fig. 7 and 8). The test product with Helioguard 365 could reduce wrinkle depth by almost 20% (Fig. 9).

Conclusion

UVA irradiation causes DNA damage. Either UVA directly harms DNA or DNA damage was caused by ROS generated by UVA irradiation.

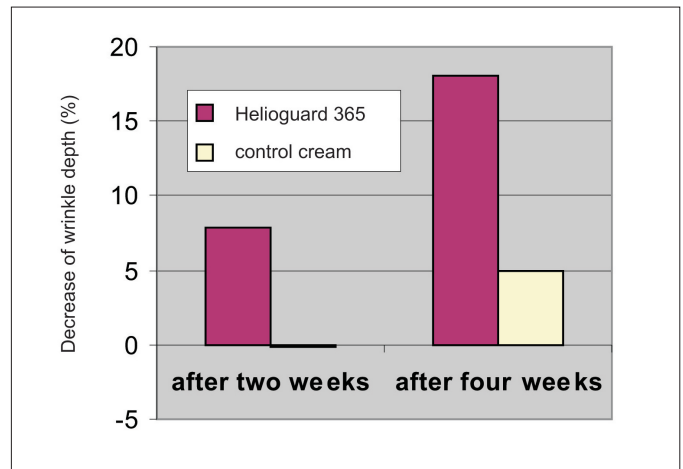


Figure 9: Wrinkles in the crow feet area are strongly reduced after a four week application of Helioguard 365

As a consequence, the metabolism of the cells becomes defective resulting in mitochondria failure, leading to further ROS generation. The viability of cells falls. In addition, the generation of matrix components such as collagen or elastin is lessened and existing matrix components are broken down by newly expressed matrix metalloproteinases. The loss of matrix components leads to thin skin and to diminished skin elasticity, promoting wrinkle development. Such changes are typical features of premature skin aging.

The experiments shown above impressively demonstrate that an extract of *Porphyra umbilicalis* containing MAA is able to reduce the UVA-caused damage to the skin. Presumably two mechanisms are relevant for this effect. MAA are able to absorb UVA radiation as they do in algae, corals and zooplankton. In this way, DNA damage is countered. Further, antioxidative substances in the extract reduce the damage caused by ROS. Hence, the daily use of Helioguard 365 seems to be a highly effective way to sustain skin smoothness and firmness as well as to prevent premature skin aging.

Literature

- 1) Rudolph T. and Traeger Ch., 2002, 22nd IFSCC Congress Edinburgh, oral presentation, volume 2;
- 2) Groeninger A., Sinba R.P., Klisch M., Haeder D.-P., 2000, Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae - a database. *J Photochem Photobiol B, Biology* 58: 115-122. ■■