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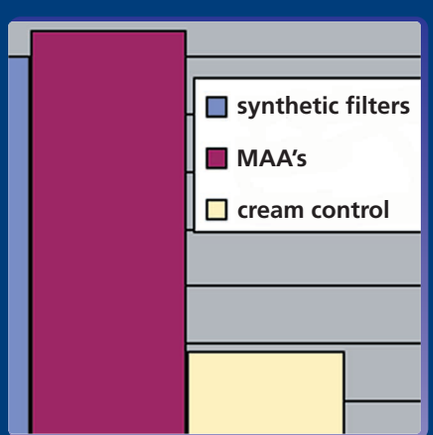

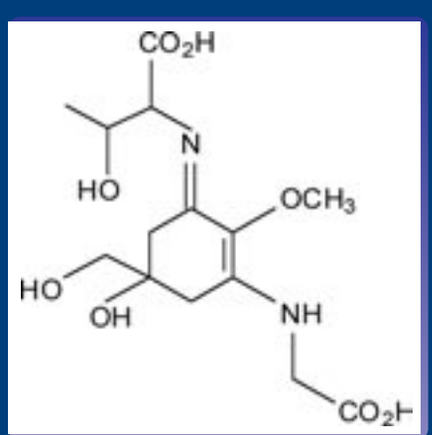

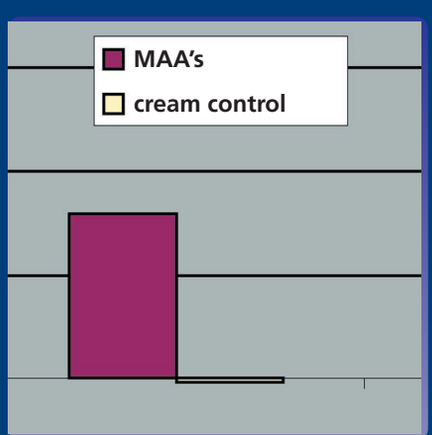

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D. Schmid, C. Schürch, F. Zülli, H.-P. Nissen, H. Prieur:

Mycosporine-like amino acids:

Natural UV-screening compounds from red algae to protect the skin against photoaging

D. Schmid, C. Schürch, F. Zülli*, H.-P. Nissen, H. Prieur**

Mycosporine-like amino acids: Natural UV-screening compounds from red algae to protect the skin against photoaging

Keywords: UVA, photoaging, Mycosporine-like amino acids, *Porphyra umbilicalis*

Abstract

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's) that are produced by certain algae, corals and cyanobacteria. The peak absorption of MAA's is in the UVA range and their absorption coefficients are similar to those of synthetic sunscreens. A human study showed that a cream containing MAA's from the red alga *Porphyra umbilicalis* efficiently protects the skin against UVA exposure on a typical working day.

Introduction

Solar UV radiation can induce acute skin reactions like erythema but produces also long term effects such as premature skin aging (photoaging) and carcinogenesis. The solar UV spectrum that 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 region can penetrate much deeper into the skin. UVA reaches the dermis where it is responsible for the premature skin aging effects of sun light.
UVA alters the expression of certain genes by generation of reactive oxygen species (ROS) and / or stimulation of pro-inflammatory cytokines such as IL-1β and TNF-α (1). It has been shown that UVA activates the transcription factors NF-κB and AP-1 (2). This results in the induction of a series of collagen and elastin degrading enzymes, the so called matrix metalloproteinases (MMP's). 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 chromophors, 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 molecule can react with proteins or with lipids. The reaction products, such as lipid peroxides, are themselves ROS and have lost cellular functionality (3).
While UVB is highly dependent on the season, day-time, cloudiness and latitude, UVA is relatively constant during the year. In middle Europe erythemal UVB is of minor importance for daily skin care products, but UVA is present all year round in harmful doses that are received indoors as well since UVA easily penetrates window glass. To estimate individual, daily UV exposure, test persons were equipped with small UV dosimeters (4). Exposure was measured on typical working days, taking into account also indoor UV radiation. Their data show that indoors UVB is in contrast to UVA not of relevance. A daily UVB dose of 2.5 MED's at open windows on a sunny summer day is reduced to 0.2 MED at closed windows. But about 40 % of the outdoor UVA radiation could be still received indoors. Of real relevance for daily skin care are therefore not SPF factors but UVA protection. Since synthetic filters might be problematic for everyday application because of endocrine side effects (5), 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's). 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's (6). The red alga *Porphyra umbilicalis* is reported to produce the MAA's Porphyra-334 **(Fig. 1)** and Shinorine (7). Their absorption coefficients (ε_{molar}) at 334 nm 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).
This article summarizes the study results on the efficacy of a cream with isolated MAA's from *Porphyra umbilicalis* to prevent photoaging induced by UVA irradiation.

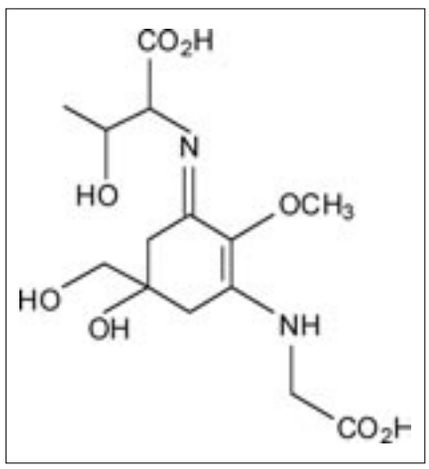

Fig. 1 Chemical structure of the mycosporine-like amino acid Porphyra-334

Materials and Methods

Extraction of MAA's
Dried *Porphyra umbilicalis* material was suspended in water at 3.3 % and ex-

tracted by incubation at 45 °C for 2 hours under constant stirring. After removal of the algae material the extract was clarified by ultrafiltration 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 was adjusted to 0.1 %.

HPLC-Analysis of MAA's
Separation of MAA's was done on a Superspher 100 RP 18 column (4 µm, 254 x 4 mm) with a LiChrospher 100 RP 18 endcapt precolumn (5 µm). The mobile phase was 0.02 % acetic acid and the flow rate 1.0 ml/min. The detection wavelength was 334 nm. Identification was done by comparison with MAA standards that were provided by Prof. Dr. *D.-P. Häder* (Friedrich-Alexander University of Erlangen, Germany).

Cell culture experiments
Mouse fibroblasts 3T3 were cultured in 24 well plates in 100% DMEM for 24 hours at 10 % confluence ($1x10^4$ cells/ml) in an incubator at 37 °C with 5 % carbon dioxide. After the medium was replaced by a mixture of 95 % basal medium and 5 % DMEM, the test substances were added. One series was irradiated with UVA for 1 min, the other series stayed untreated as control. After 48 hours incubation, the cells were fixed in 100 % methanol and stained in Coomassie blue staining solution. For determination of cell number, the absorption at 580 nm was measured.

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 (GFMesstechnik 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 standard cream with 5 % MAA test substance (final MAA concentration of 0.005 %) and a standard cream with 4 % Neo Heliopan AV and 1 % Parsol 1789. The standard cream without actives served as control. The test products were applied twice daily on the inner side of the forearm and on the face (only the cream containing MAA's to measure depth of wrinkles). The test areas on the forearm were irradiated twice weekly with UVA (10 J/cm²).

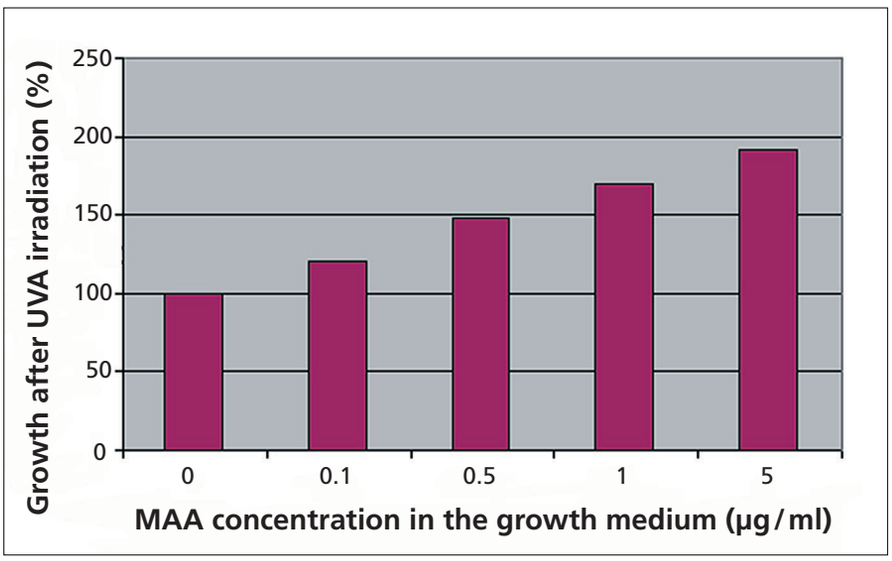

Fig. 2 Protection of fibroblast cells by mycosporine-like amino acids against UVA

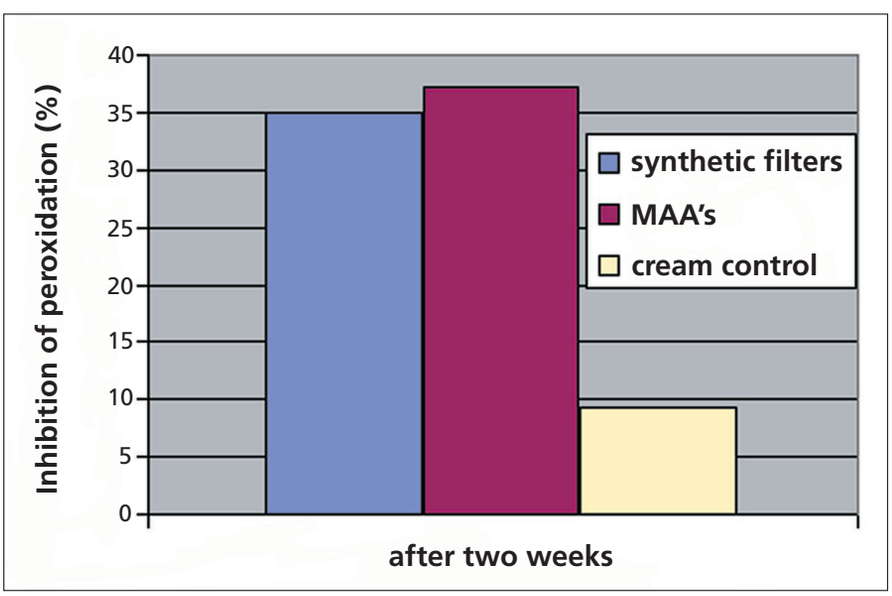

Fig. 3 Inhibition of lipid peroxidation compared to the beginning of the study and the untreated area

Results and Discussion

Analysis of *Porphyra umbilicalis*
Analysis by HPLC showed that *Porphyra umbilicalis* contained the MAA's Porphyra-334 and Shinorine, in a ratio of 11.5 : 1. The total MAA concentration was 1.4 % of dry mass. This is considerably more than the reported typical concentration range of 0.16 to 0.84 % (6).

Cell culture experiments
After UVA irradiation, growth of fibroblast cells was reduced compared to non-treated cells. The presence of 0.1 to 5 µg/ml MAA's in the culture medium resulted in a concentration-dependent protection against the growth-limiting UVA irradiation **(Fig. 2)**. The

not-irradiated control assays showed no effect of MAA's in the concentration range of 0.1 to 5 µg/ml. These fibroblast cell culture assays demonstrate a specific UVA protection effect of MAA's. The effect was measurable already at 0.1 µg/ml MAA's (0.00001 %) in the culture medium.

Human study

Normal UVA exposure at non-protected skin areas is between 3 and 20 J/cm². *Rudolph* and *Träger* (4) measured an UVA exposure of 8 J/cm² at the outside of the forearm that is facing the window, during a day that was cloudy and sunny. The irradiation dose that 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 mimics a daily photoaging of the skin, and its protection with creams.

The study results demonstrate that both test products could reduce the primary injure of UVA irradiation such as lipid peroxidation by about 35 % **(Fig. 3)**. Application of the standard cream without actives could minimally inhibit (10 %) lipid peroxidation. Already after two weeks application the test products significantly reduced the secondary effects of UVA exposure shown by improved skin conditions such as firmness and smoothness. After four weeks these parameters were improved by more than 10 % in areas treated with the MAA test cream and by about 6 % in zones treated with synthetic filters (**Fig. 4 and 5**). The MAA test product could reduce wrinkle depth by almost 20 % **(Fig. 6)**.

Conclusion

The study clearly demonstrates that a cream with 5 % MAA test substance can neutralize the UVA effects as efficient as a cream with 1 % synthetic UVA filters and 4 % UVB filters. The UVB sunscreens are not of relevance in this study as only UVA irradiation was tested. But as outlined in the introduction this corresponds to normal working day situations in middle Europe where only UVA effects are important. Considering the UVA-filtering capacity, the cream with 1 % synthetic UVA filter is 200 times more active than the MAA test product (0.005 % MAA's). The fact that this was not reflected in the study results (or even in the opposite way) might indicate that the application of 5 % UV-filters is by far more than needed for an efficient protection against photoaging during normal working days. Thus, MAA's are a welcome, natural alternative to protect our skin against daily UVA exposure (8.).

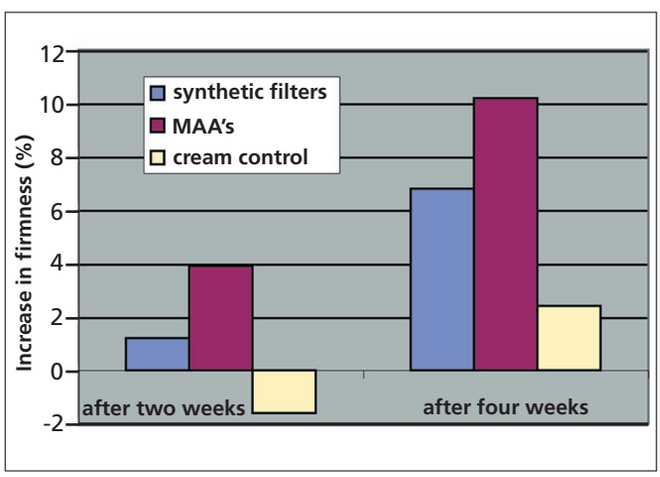

Fig. 4 Improvement of skin firmness compared to the beginning of the study and the untreated area

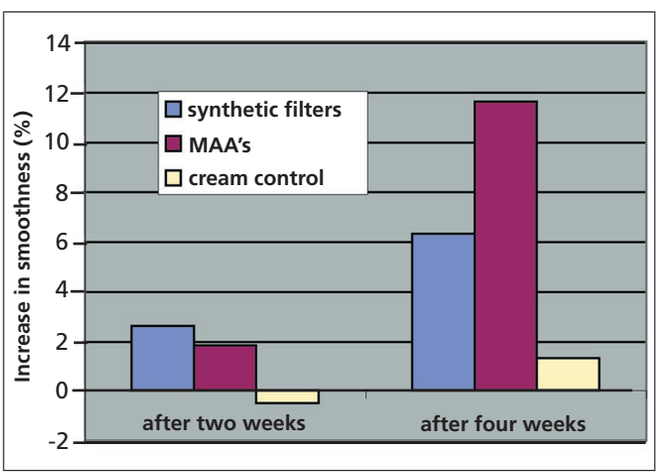

Fig. 5 Improvement of skin smoothness compared to the beginning of the study and the untreated area

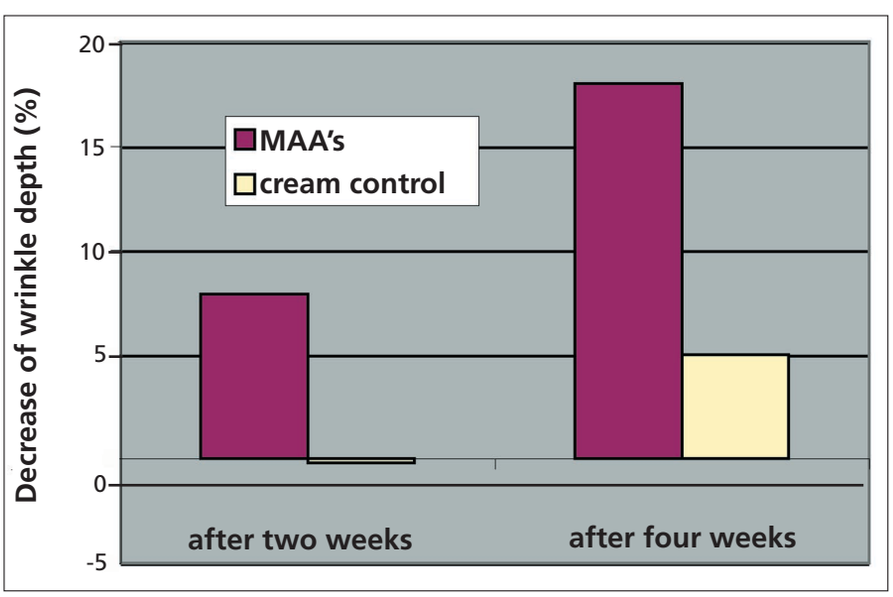

Fig. 6 Reduction of wrinkle depth compared to the beginning of the study and the untreated area

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