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UVA-screening compounds from red algae protect against photoageing

INTRODUCTION

Mycosporines were first described as fungal metabolites that strongly absorb in the UVB range, maximally at 310 nm (Leach). They were initially assumed to function as differentiation or reproduction markers but later postulated to protect fungal spores against UV radiation. Mycosporines are the parent compounds to the mycosporine-like amino acids (MAAs) that are found in aquatic organisms such as cyanobacteria, red algae, dinoflagellates and corals but also in terrestrial cyanobacteria (for the chemical structure see Fig. 1). Whereas mycosporines contain exclusively oxo-carbonyl chromophores, most of the MAAs contain imino-carbonyls that absorb the short wavelengths of the UVA (Shick & Dunlap). Incorporation of various amino acids or iminoalcohol groups results in a diversity of about 20 MAAs (Cockell & Knowland).

MAAs are the strongest naturally occurring UVA-absorbing compounds. Their primary function seems to be the screening of UV-radiation, because their expression is inducible by UV-light. Also their localisation argues for a screening role. In single cell organisms the water-soluble MAAs are distributed homogeneously in the cytoplasm and in multicellular organisms they are localised at the surfaces and are easily extracted. Corals that live in clear shallow water, a UV-intense environment, are found to produce 13 different MAAs (Shick & Dunlap). Mountain lakes are another ecosystem with high UV-stress, because exposure to UV increases with altitude and because the water is normally very clear so that UV-rays can penetrate to depths of 20 m. Seven different MAAs were detected in the zooplankton of mountain lakes in the Central Alps (Sommaruga).

The red alga *Porphyra umbilicalis* lives in all oceans at the shore area on rough-surfaced rocks. It is a small alga, up to 20 cm, greenish when young and later purplish-red, with an irregularly shaped frond that is membranous but tough. *P. umbilicalis*, commonly known as Purple Laver in America and Europe or as Nori in Asia, is the most widely consumed seaweed in the world. *P. umbilicalis* is reported to produce the MAAs Porphyra-334 (Fig. 1) and Shinorine (Gröniger *et al*). Their absorption coefficients (ε_{molar}) at 334nm are 42'300 and 44'700 respectively (for the spectrum see Fig. 2). Their filter capacity is therefore similar to that of synthetic UVA sunscreens such as Butyl Methoxydibenzoylmethane (ε_{molar} 40'000) and Terephthalylidene Dicamphor Sulfonic Acid (ε_{molar} 45'000).

UVA is regarded as the main cause of premature skin ageing. In contrast to UVB, UVA is present all year round in harmful doses that are received indoors as well since UVA easily penetrates window glass. In temperate climate zones, erythemal UVB is of minor importance for daily skin care products because UVB is highly dependent on the season, time of day, cloudiness and latitude. Of real relevance for daily skin care are therefore not SPF factors but UVA protection. It is therefore recommended to use day creams with UVA protection ingredients. Although the percutaneous absorption of synthetic UVA sunscreens is low (Benech-Kieffer *et al*), there will be a substantial systemic accumulation when they are used daily, because day creams with UV-protection contain up to 1 to 5% pure synthetic UVA sunscreens. Natural UV-absorbing substances are therefore a well perceived alternative for daily skin care.

SUMMARY

Whereas the intensity of UVB depends on the season, the time of day and cloudiness, UVA is present every day in significant amounts, even indoors. Of real relevance for daily skin care is therefore not the SPF-value but the efficacy of the product to protect against UVA. Mycosporine-like amino acids are powerful, natural UVA screening substances produced by certain algae, corals and cyanobacteria. The food grade red alga Porphyra umbilicalis *was used to isolate mycosporine-like amino acids. In cell culture experiments with mouse fibroblasts isolated mycosporine-like amino acids were found to protect in a concentration dependent manner against UVA irradiation. The mycosporine-like amino acids were then tested in a human study with exposure to UVA doses corresponding to typical working day situations. The study showed that a product with mycosporine-like amino acids performed as well as a cream with a synthetic UVA sunscreen regarding skin lipid oxidation and skin ageing parameters such as elasticity and roughness.*

Materials and methods

Extraction of MAAs

Dried *Porphyra umbilicalis* material was suspended in water and extracted by incubation at 45°C for two 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 MAAs

Separation of MAAs was done on a

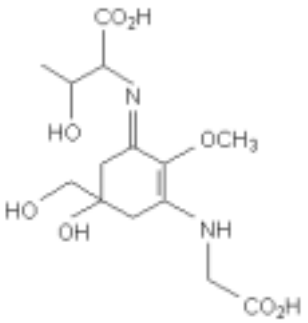

Figure 1: *Chemical structure of the mycosporine-like amino acid Porphyra-334.*

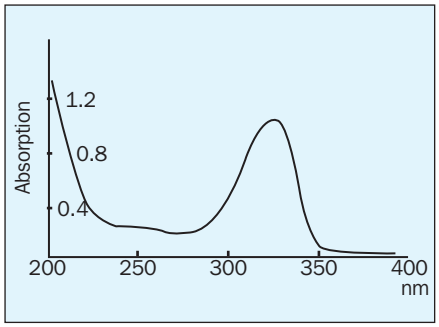

Figure 2: *Absorption spectrum of the MAAs isolated from the red alga Porphyra umbilicalis.*

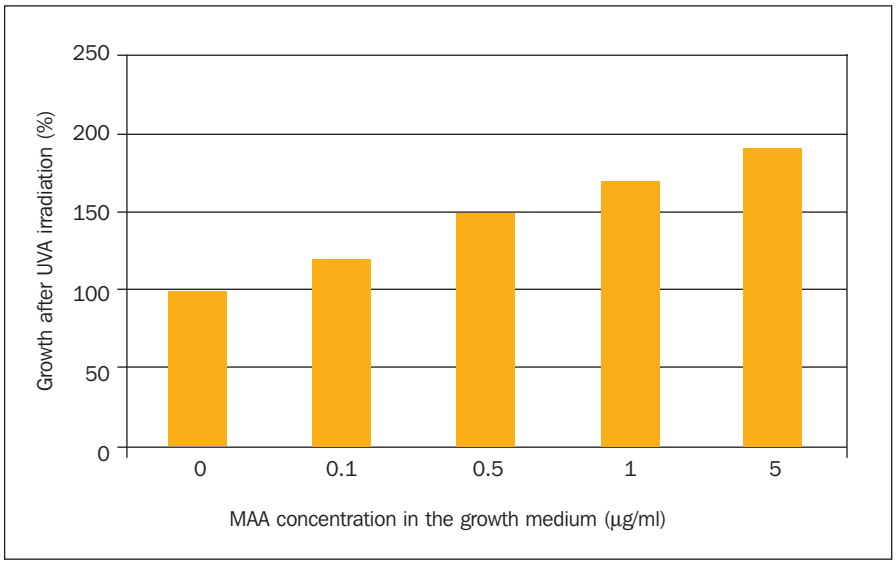

Figure 3: *Protection of fibroblast cells by mycosporine-like amino acids against UVA.*

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 range of 36 to 54, the following skin parameters were analysed: 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% Ethylhexyl Methoxycinnamate and 1% Butyl Methoxydibenzoylmethane. The standard cream without actives served as control. The test products were applied twice daily on the inner side of the forearm. The test areas were irradiated twice weekly with UVA (10 J/cm²).

Results

Analysis of Porphyra umbilicalis

Analysis by HPLC showed that *Porphyra umbilicalis* contained the MAAs Porphyra-334 and Shinorine, in a ratio of 2:1. 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% (Cockell & Knowland).

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 MAAs in the culture medium resulted in a concentration-dependent protection against the growth-limiting UVA irradiation (Fig. 3). The not-irradiated control assays showed no effect of MAAs in the concentration range of 0.1 to 5 µg/ml. These fibroblast cell culture assays demonstrate a specific UVA protection effect of MAAs. The effect was measurable already at 0.1 µg/ml MAAs (0.00001%) in the culture medium.

Human study

The irradiation dose that was applied in this study (two times 10 J/cm² per week) corresponds to an UVA exposure at a non-protected skin site in temperate climate zones on days with cloudy and sunny periods and mainly indoor activities (Rudolph & Träger). Thus, the study mimics UVA stress typical for working day situations where protection can be provided by daily skin care products.

The results show that both test products could reduce lipid peroxidation, the primary injure of UVA irradiation by about 35% (Fig. 4). Lipid peroxidation was also slightly reduced by application of the standard cream without actives. The test products influenced also positively the secondary effects of UVA exposure, firmness and smoothness. Already after two weeks these parameters were found to be significantly improved by application of the test products compared to the zones were no cream or the standard cream without actives were applied (Fig. 5 and 6). After four weeks skin firmness and smoothness improved by more than 10% in zones treated with the MAA test cream and by about 6% in zones treated with synthetic filters.

Discussion

The study clearly demonstrates that a cream with 0.005% MAAs can neutralise 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 because the test areas were only exposed to UVA irradiation. But as outlined in the introduction, this corresponds to normal working day situations in temperate climate zones 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. The fact that this was not reflected in the study results indicates that the absorption capacity is not the only factor important for protection of the skin against photoageing. Highly important is what happens to the screening compounds when they switch energy levels during absorption. The ideal sunscreen rapidly and effectively transforms absorbed UV

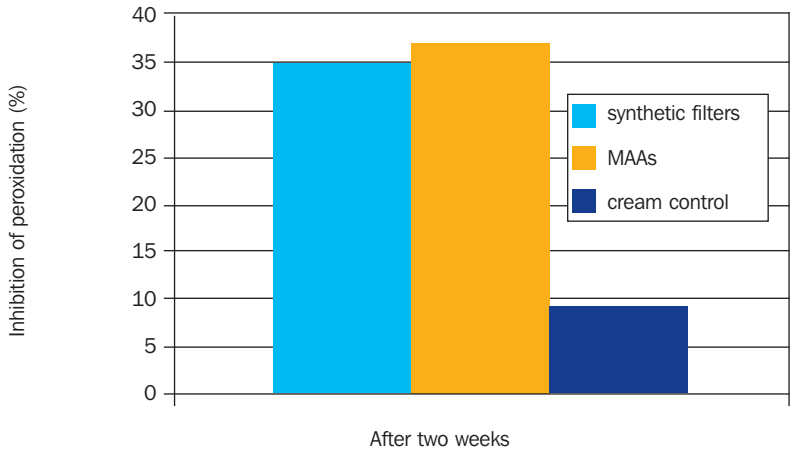

Figure 4: *Inhibition of lipid peroxidation compared to the beginning of the study and the untreated area.*

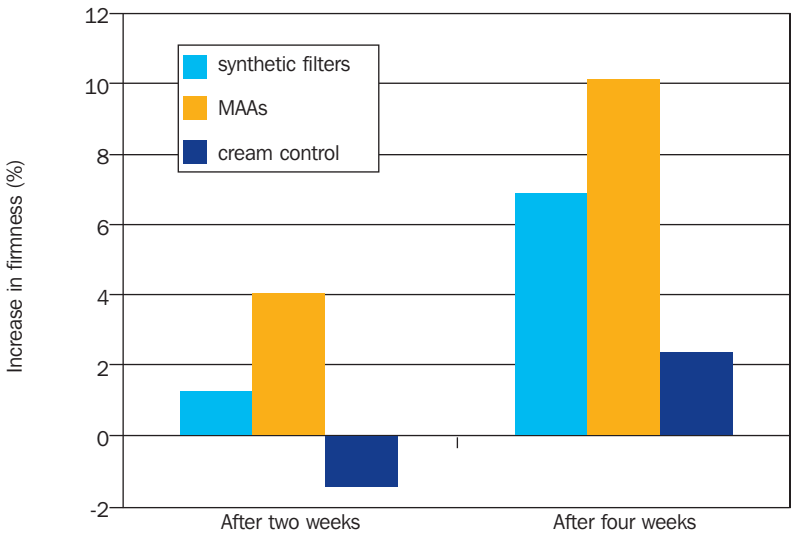

Figure 5: *Improvement of skin firmness compared to the beginning of the study and the untreated area.*

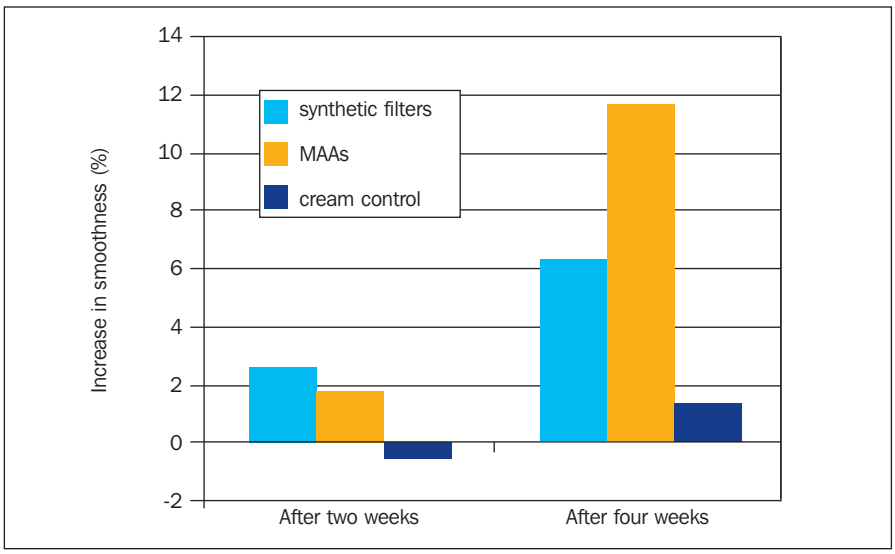

Figure 6: *Improvement of skin smoothness compared to the beginning of the study and the untreated area.*

into harmless thermal energy with no subsequent loss of protective power. But some synthetic UV filters are reported to generate free radicals when illuminated (Damiani *et al*.) or have been shown to partially decompose into inactive reaction products upon UV exposure (Chatelain & Gabard). Analysis of the photophysical and photochemical properties of MAAs showed their qualification as photo protectors because they did not produce reactive intermediates upon irradiation (Conde *et al*). Antioxidants present in the MAA extract might have also exerted a protective role in the photoageing study. One of these antioxidants could be the MAA precursor molecule in the biosynthesis, 4-deoxygadusol, that was found to have strong antioxidant properties (Dunlap *et al*). PC

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