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D. Schmid, F. Züllli:

**Cosmetic Actives for an Efficient  
Treatment of Age Spots**

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# Cosmetic Actives for an Efficient Treatment of Age Spots

## Introduction

Age spots, also known as sun- or liver spots, are brown to black macules that are common on sun-exposed skin after the age of 40. Age spots are harmless and do not really need treatment, but they are a significant visual clue to a woman's age. This was clearly shown in a study where digital imaging technology was used to standardize female faces in form and surface topography. Rating of a set of standardized faces, varying only in age- and photodamage-induced skin color distribution, revealed that age

spots have a major influence on the perception of female facial age (1). Two types of pigments are present in age spots, the melanins and lipofuscin. They are overproduced in the spot area because of an overreaction to UV radiation. UV light leads to the generation of free radicals and reactive oxygen species in keratinocytes. This induces the formation of signaling molecules like the  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), the endothelin-1 (ET-1) and the stem cell factor (SCF), and leads also to the oxidation of proteins and lipids (Fig. 1). Oxidized proteins and lipids form insoluble, dark pigmented complexes, called lipofuscin. The released signaling molecules bind to their corresponding

receptors on melanocytes where they stimulate melanin production and promote dendrite formation. The messenger molecules ET-1 and SCF are shown to be highly overproduced in age spot areas (2). This explains the higher melanin content compared to the neighboring, normally pigmented skin. Accumulation of oxidized proteins and thus lipofuscin formation is normally prevented by the proteasome system. It is a complex of proteases that specifically recognizes damaged proteins and then degrades them completely. But proteasome activity is known to decline with advancing age (3), explaining why uneven pigmentation is a typical symptom of old age.

## Abstract

**A** test cream containing as active ingredients a combination of the soy isoflavone genistein with a cress sprouts extract standardized in sulforaphane was found to be very efficient in the treatment of age spots. This implies that the actives worked mainly in the spot area and did not induce a strong bleaching effect on the neighboring, normally pigmented skin. The targeted efficacy is probably the result of blocking the overreaction of skin to sun light that is typical for age spots and of preventing lipofuscin formation.

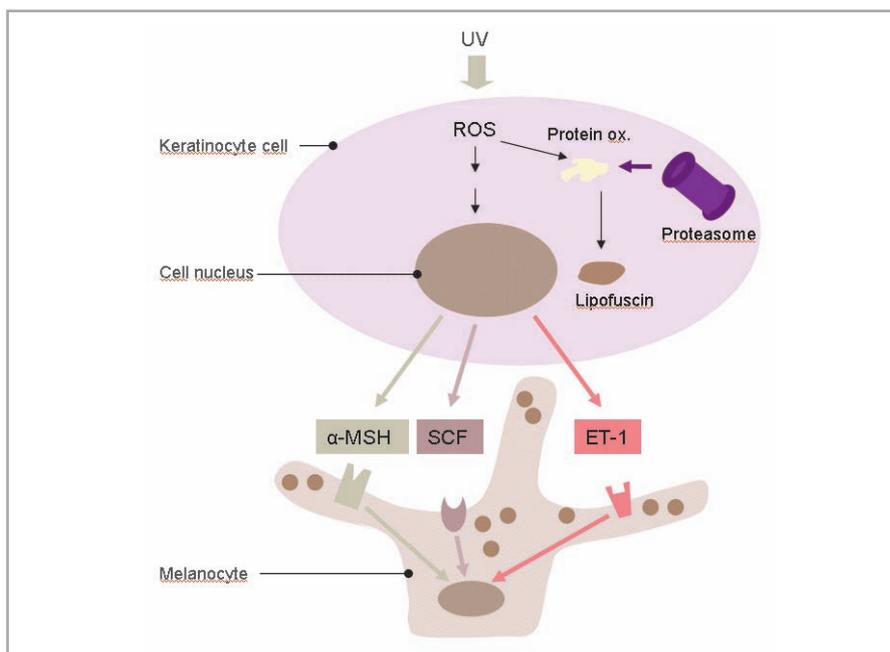


Fig. 1 Signaling mediators involved in the formation of lipofuscin and melanin in age spots

■ Active Ingredients for Efficient and Specific Treatment of Age Spots

The cosmetic treatment of age spots is normally a combination of the regular use of sunscreens for prevention, and application of whitening products to fade the spots. But, if not applied only on the spot area, the fading effect is minimal because this kind of treatment will whiten the skin all over. And as most of the women still prefer a slight, healthy-looking tan, they are looking for a product that specifically treats age spots. This article describes a cosmetic ingredient that meets these criteria. The ingredient is based on a combination of the soy isoflavone genistein with a cress sprouts extract.

Genistein is a well known natural inhibitor of the tyrosine kinase, an enzyme involved in several signaling cascades from receptors at the cell surface to regulators of gene expression. A tyrosine kinase is reported to be at the intracellular side of the SCF receptor (4). There are also reports about the involvement of tyrosine kinase in the ET-1 signaling. Thus, genistein inhibits the effects of SCF and probably also of ET-1 on melanocytes. In this way, genistein regulates the high concentration of SCF and ET-1 typically found in age spots.

The cress sprouts extract helps in several ways against age spots. Cress sprouts are a rich source of the isothiocyanate sulforaphane. Isothiocyanates are sulfur-containing chemicals that are characteristic of the Brassicaceae family. Well known members of this family include broccoli, rapeseed, mustard, radish and cress. Isothiocyanates give these vegetables their typical pungent taste and are produced to repel herbivores. Sulforaphane works as an indirect antioxidant (5). Direct antioxidants such as the vitamins C and E can neutralize an oxidant once and need then to be replenished by other antioxidants. Indirect antioxidants work by expressing a series of genes that code for cytoprotective proteins. These proteins are enzymes that synthesize or regenerate a lot of different direct antioxidants. Enzymes work catalytically, meaning that they are able to do thousands of working steps with-

out being consumed. In this way, sulforaphane can be used to neutralize free radicals and reactive oxygen species that represent the first UV-induced triggers for formation of lipofuscin and melanins. The cress sprouts extract was also found to inhibit the  $\alpha$ -MSH-induced melanin synthesis. This was shown in a cell-based assay using B16 murine melanoma cells. Cultivation was done in 96 well-plates for 72 hours in the presence of a stable derivative of  $\alpha$ -MSH. After incubation, the melanin content was analyzed by measuring the optical density at 405 nm. A plate that was cultivated in parallel was used for the evaluation of cell viability by the MTT assay. The cress sprouts extract was tested at three different concentrations. Melanin formation was strongly inhibited at 0.4% (Fig. 2). The MTT assay clearly demonstrated that this was not the consequence of a cytotoxic effect. The inhibitory effect of the extract on melanin formation after stimu-

lation with  $\alpha$ -MSH could also be demonstrated with normal human melanocytes. The cress sprouts extract at 0.016% reduced melanin synthesis by 47%. The cress sprouts extract was not active in assays with isolated human tyrosinase. The results of the cell-based assays with B16 cells or the normal human melanocytes therefore indicate that the cress sprouts extract reduces the binding of  $\alpha$ -MSH to its receptor on melanocytes.

The cress sprouts extract turned out to be very active in a cell-based screening assay for modulators of the proteasome system. The Proteasome-Glo™ Assay (Promega) was used to analyze the effects on the proteasome activity in normal human dermal fibroblast cells. The assay is based on a proteasome substrate labeled with aminoluciferin. This coupled-enzyme system, with simultaneous proteasome cleavage of substrate and luciferase consumption of the released aminoluciferin, results in a luminescent

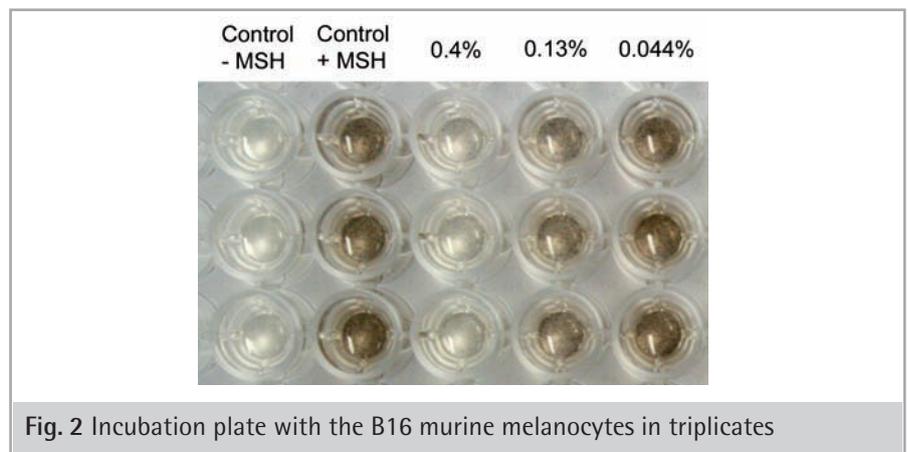


Fig. 2 Incubation plate with the B16 murine melanocytes in triplicates

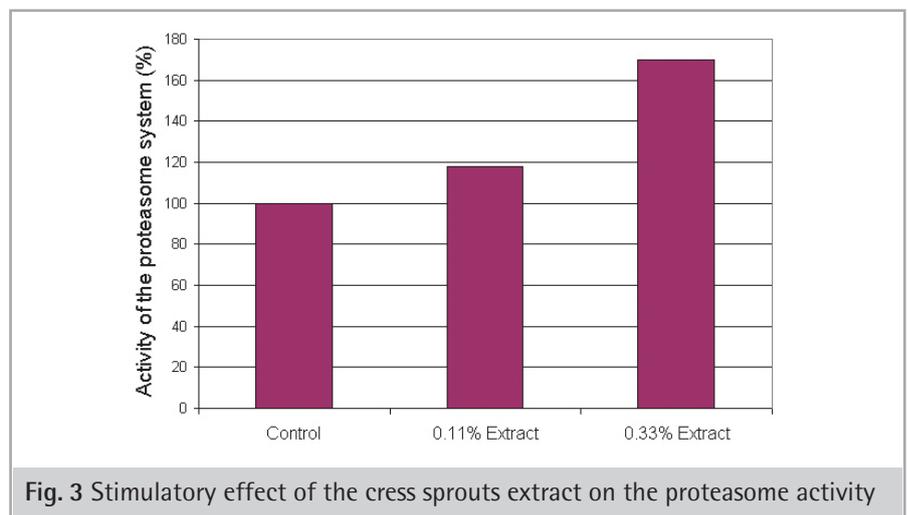


Fig. 3 Stimulatory effect of the cress sprouts extract on the proteasome activity

signal that is proportional to the proteasome activity. Four hours after incubation with 0.33% of the cress sprouts extract, the proteasome activity was found to be 70% increased (Fig. 3). By stimulating the proteasome activity, the cress sprouts extract can prevent or reduce the accumulation of oxidized proteins and thus inhibit lipofuscin formation.

■ The Efficacy against Age Spots in Clinical Study

A cream containing 60 mg/kg genistein, encapsulated into lecithin liposomes, and 2% of an aqueous cress sprouts extract was tested. The concentration of sulforaphane in the extract was standardized to 100 µM. The study was performed with 10 women aged between 47

and 77. The test cream was applied twice daily for four weeks to defined spots as well as to defined normally pigmented skin areas on one hand. The placebo cream was applied in similar way to the other hand. For analysis of skin pigmentation, the melanin index was measured with the Skin Pigmentation Analyzer® SPA99 (Courage & Khazaka) at the beginning of the study and after four weeks. The study results showed that the test cream could significantly fade the age spots (Fig. 4). After four weeks' application and compared to age spots treated with the placebo cream, the melanin index was reduced by 6.2%. There was no difference in the melanin index in the normally pigmented skin between the test cream and the placebo. The effect of the test cream on age spots could also be shown on digital photos (Fig. 5).

■ Conclusions

Age spots are caused by a local overreaction to sun light. The result is an increased formation of two types of pigments, namely melanin and lipofuscin. For an efficient treatment, both types of pigments have to be inhibited in their synthesis. Genistein, a known tyrosine kinase inhibitor, is supposed to interfere with the SCF and ET-1 cytokine signaling between keratinocytes and melanocytes that were shown in literature to be overactive in age spots. The cress sprouts extract blocks the activity of the sun light induced paracrine factor α-MSH. All these activities result in a reduced formation of melanin. The cress sprouts extract acts as a promoter of the cell's own defense against oxidative stress and as a specific stimulator of the proteasome system reducing the formation of lipofuscin.

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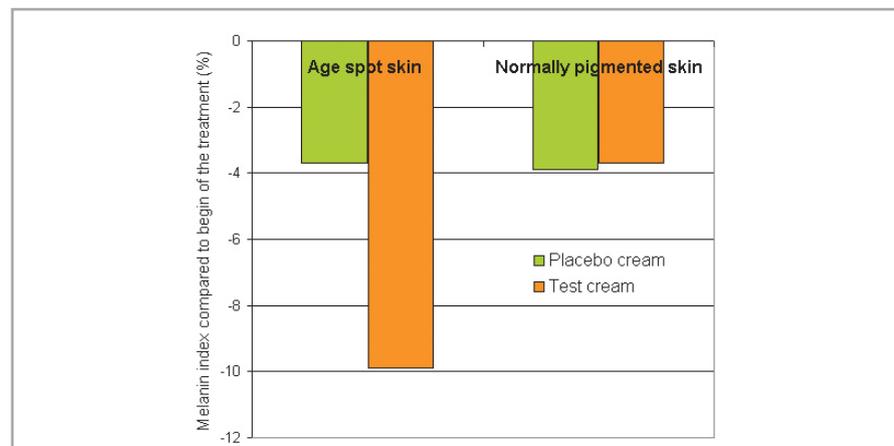


Fig. 4 The effects of the test creams on melanin index after four weeks' application

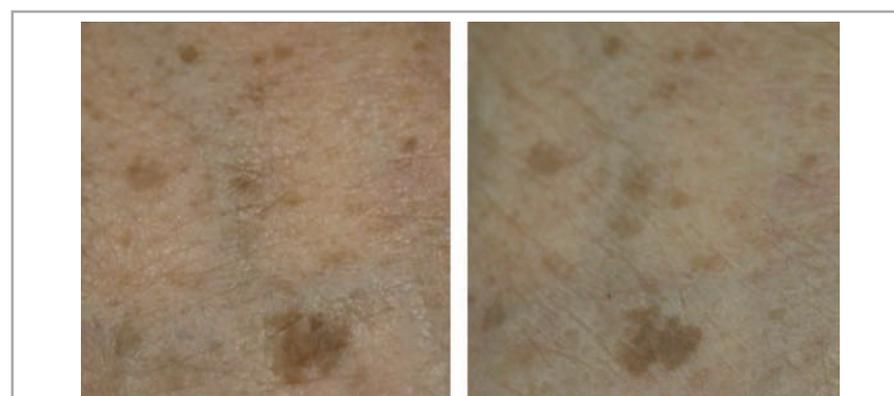


Fig. 5 Photos of age spots on the hand of one subject, before (left) and after (right) treatment

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