Cress Sprout Actives to Turn on the Master Switch in Cell Protection

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Abstract

The protein Nrf2 plays a central role in the cellular defense system against harmful environmental toxicants. It turned out also to be a key regulator in the UV response of the skin. The plant compound sulforaphane, naturally present in Brassicaceae vegetables, is a well known inducer of Nrf2. Garden cress sprouts were used to prepare a sulforaphane-containing cosmetic ingredient. Tested on human keratinocytes, the ingredient was found to strongly stimulate the synthesis of enzymes that detoxify oxidants and electrophiles. In clinical studies, the ingredient showed a general skin brightening effect and, in combination with the soy isoflavone genistein, protection of the skin against the formation of uneven pigmentation.

Introduction

Free radicals generated by oxidative stress and electrophilic, toxic compounds represent the main threat at the cellular level. If persistent, this cellular stress will lead to tissue damage. Our cells respond to these toxic chemicals by increasing the synthesis of cytoprotective proteins. These are detoxifying proteins and enzymes that rebalance the redox status by neutralizing electrophiles and replenishing used cellular antioxidants such as glutathione. These cytoprotective proteins are characterized by a specific gene sequence, called antioxidant response element (ARE). The expression of these proteins is regulated by the transcription factor Nrf2 that binds to the ARE site in the promoter regulatory sequence (1). Under basal conditions, Nrf2 is largely bound in the cytoplasm to Keap1. As a heterodimer, Nrf2 is inactive. In response to toxic chemicals and oxidative stress, the Nrf2-Keap1 complex is disrupted and Nrf2 translocates into the cellular nucleus where it binds to the ARE sequences to induce the expression of cytoprotective proteins (2). Nrf2 thus plays a central role in cellular protection. Recent scientific publications also demonstrate for Nrf2 a key role in the UV response of the skin. UVA, that is known to generate a significant oxidative stress, was found to induce the expression of the protecting, ARE-containing enzyme heme oxygenase 1 (HO-1) (3).

Sulforaphane is the best described, natural activator of Nrf2 (4). It is a sulfur-containing compound (fig. 1) that is typical for vegetables of the Brassicaceae family and is responsible for the health benefits of these vegetables. Well known members include

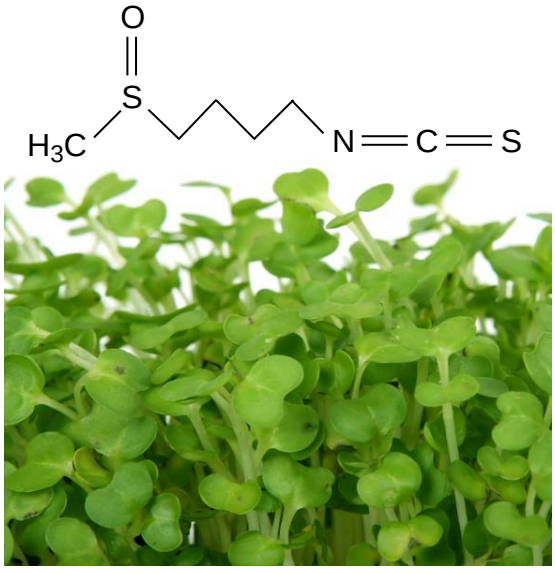

Figure 1 Molecular structure of sulforaphane

cabbage, broccoli, cauliflower, kale, rape-seed, mustard, radishes, horse radish, water and garden cress. Sulforaphane gives them a spicy aroma and a refreshing, peppery-pungent taste. The sprouts of these vegetables contain the highest concentration of sulforaphane. In broccoli sprouts that have been found to exert cancer-preventive effects, the concentration of sulforaphane is 20 to 50 times higher than in mature broccoli (5). A recent publication demonstrated the protective effects of sulforaphane against damage by UV radiation (6). The article showed that topically applied sulforaphane reduced susceptibility to erythema by

mobilization of the cellular enzymatic defense system. The mechanism of action of sulforaphane is based on specific modifications of critical cysteine residues of Keap1 which leads to the liberation of Nrf2 (7) (fig. 2).

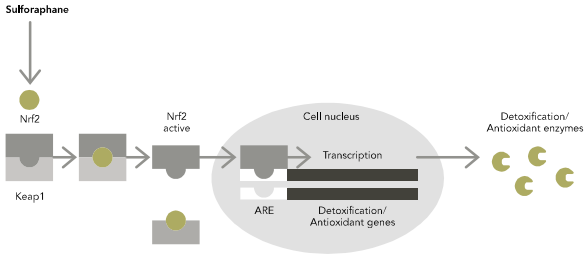

Figure 2 Induction of the expression of cytoprotective enzymes by sulforaphane

This paper shows results of cell-based assays and clinical studies with a cosmetic ingredient containing sulforaphane (tradename: SulforaWhite). Garden cress sprouts were used as the raw material to produce the ingredient. For a better skin uptake, the actives of the cress sprout ingredient were incorporated into liposomes.

Materials and Methods

1. Preparation of a cress sprout ingredient
Garden cress is suitable for hydroponic cultivation and is typically harvested just a few days after germination. 4 to 5 day old garden cress sprouts were used as the raw material to produce the ingredient. The INCI composition is: Lepidium Sativum Sprout Extract, Glycerin, Lecithin, Phenoxyethanol and Aqua. In the sprouts, sulforaphane is present as a glycoside, called glucoraphanin. For analysis of the sulforaphane content, the sprout extract was first treated with myrosinase to hydrolyze the glycosides and the resulting sulforaphane was measured by HPLC after cyclocondensation with 1,2-benzenedithiol (8). The concentration of sulforaphane in the final ingredient was standardized at 18 mg/l.
The ingredient used for the study on uneven skin pigmentation was a mixture of the sulforaphane ingredient with a solution of the soy isoflavone genistein (tradename: Delentigo). The final genistein concentration was 1.4 g/l and that of sulforaphane was 6.6 mg/l.

2. Stimulation of the expression of cytoprotective enzymes
The capacity of the sulforaphane ingredient to modulate the expression of cytoprotective enzymes was analyzed in vitro using normal human epidermal keratinocytes. The method of real-time polymerase chain reaction (PCR) was used to measure the expression of the following genes: NADPH:quinone reductase 1 (NQO1), heme oxygenase 1 (HO-1), thioredoxin reductase 1 (TrxR1) and glutathione peroxidase (GPX1). The keratinocytes were grown in standard growth medium to 80 % confluence. Then the cells were incubated for 24 hours with 0.05 or 0.2 % cress sprout ingredient. After incubation, the cells were harvested and total RNA was extracted.

3. Whitening study on Asian skin
A human clinical trial was conducted on 22 Asian subjects aged between 22 and 39. A cream with 2% cress sprout ingredient was applied twice daily for 56 days on the inner side of one forearm. The other forearm was treated with the placebo cream. The upper arm was used as an untreated zone. Skin color was measured with the chromameter MINOLTA type CR300. Whitening is shown by increased skin clarity, measured as lightness L*, and by an increased Individual Typological Angle (ITA) degree. For illustration of the visual effects macrophotographs were taken.

4. Study on uneven skin pigmentation
A cream with 4% cress sprout / genistein ingredient was tested in a study over 8 weeks on 12 women aged between 40 and 66. The test cream was applied twice daily 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 8 weeks.

Results and Discussion

The capacity of the cress sprout ingredient to promote the expression of cytoprotective proteins was studied in normal human keratinocytes using quantitative PCR for expression analysis (fig. 3). NADPH:quinone reductase 1 (NQO1) is a major anti-carcinogenic enzyme with a principal role in transforming quinones into stable hydroquinones. Heme oxygenase 1 (HO-1) is induced after exposure to oxidative stress, such as UV irradiation or hyperoxia, indicating its role in cellular defense. Thioredoxin reductase 1 (TrxR1) works together with NADPH to control the redox balance of the cell. Compared to the untreated control, the antioxidant enzyme NQO1 was moderately stimulated by the cress sprout ingredient. HO-1 and TrxR1 were both stimulated strongly. Glutathione

peroxidase (GPX1) that has a major role in the reduction of lipid peroxides and of free hydrogen peroxide, did not respond to the cress sprout ingredient in this study.

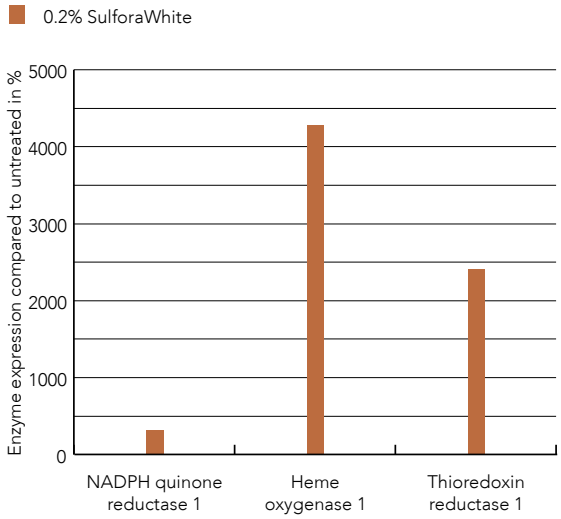

Figure 3 Effect of a cress sprout ingredient on the expression of cytoprotective enzymes

The process from exposure to UV light to pigmentation is very complex and contains many steps. As shown in figure 4, UV light leads to the generation of free radicals and reactive oxygen species in keratinocytes.

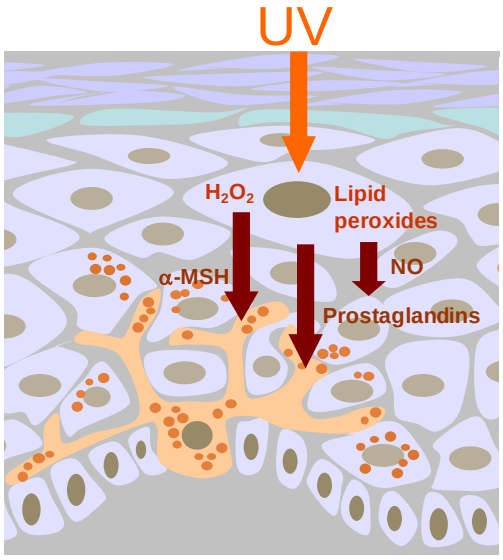

Figure 4 Signalling mediators involved in the tanning response

The primary free radicals superoxide anion $O2^{\bullet -}$, the hydroxyl radical $^{\bullet}OH$ and the secondary lipid peroxide radicals cause the keratinocytes to release inflammatory mediators such as prostaglandins and nitric oxide NO and the α-melanocyte stimulating hormone (α-MSH). Prostaglandins and α-MSH bind to their receptors on melanocytes where they induce the production of melanin pigments. Melanin is produced in specialized organelles, called melanosomes. These organelles are gradually filled with pigments, transported to the peripheral dendrite tips and transferred to the surrounding keratinocytes. There melanosomes form a protective shield around the cell nucleus, producing a uniform skin color.

If one would enhance cellular detoxification of free radicals and reactive oxygen species, then less pigmentation mediators would be produced by keratinocytes during exposure to UV light. Stimulation of cytoprotective proteins by topical application of the cress sprout ingredient should therefore lead to skin lightening. This could be demonstrated in a study on Asian skin type. The chromameter results showed a clear placebo-controlled whitening effect (fig. 5). After 56 days of use, and compared to the placebo product, the cream with the cress sprout ingredient induced a significant increase in lightness L* (+ 0.5 ± 0.2 A.U.; p = 0.004) and a significant increase in the ITA° parameter (+ 1.4 ± 0.4 A.U.; p = 0.004).

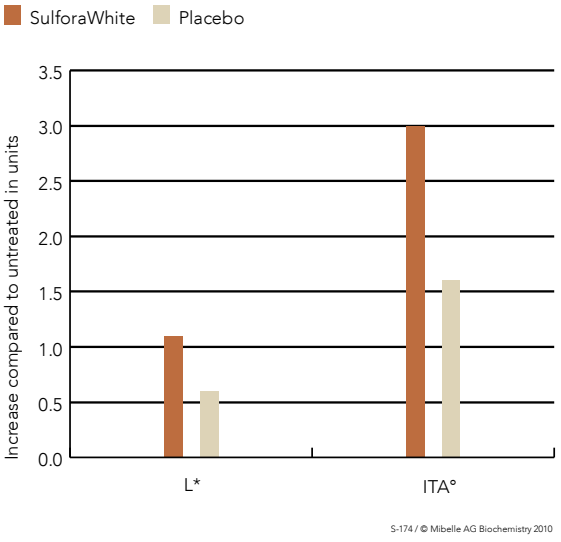

Figure 5 Increase in lightness parameter L* and ITA° value after 56 days

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 (9).

Age spots are normally treated with whitening products or chemical peels. But, if not applied only to the spot area, the fading effect is minimal because bleaching creams and peelings 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. We looked therefore for an ingredient with a targeted effect. In age spot areas, the keratinocytes were shown to highly overproduce endothelin-1 (ET-1) and the stem cell factor (SCF) (10). These are also pigmentation messengers that bind to receptors on melanocytes where they induce the production of melanin pigments. This explains the higher melanin content compared to the neighboring, normally pigmented skin. In order to get a more targeted effect on age spots, the cress sprout ingredient was mixed with the soy isoflavone genistein. It 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 (11). 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.

After 8 weeks' application, a cream with 4% cress sprout / genistein ingredient was found to clearly fade age spots (fig. 6).

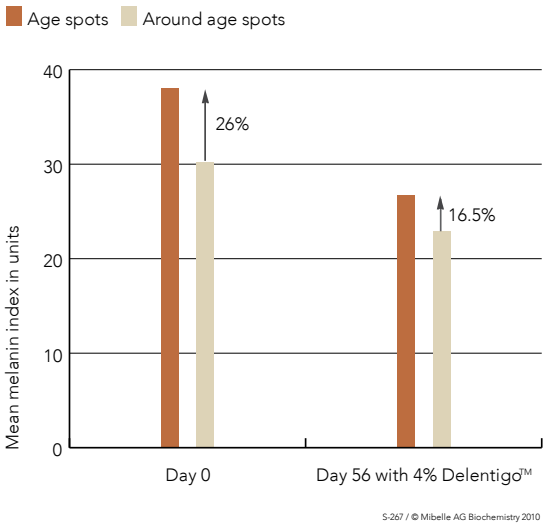

Figure 6 Effect of a cream with the cress sprout / genistein ingredient on melanin index after eight weeks' application

It was possible to demonstrate a better effect of the test cream on the spot zone compared to the neighboring, normally pigmented skin. The 26% higher melanin index of the age spot, measured before application, could be reduced to a difference of only 17% after 8 weeks' treatment.
Also on digital photos one could easily see that the age spots were less prominent after the treatment (fig. 7).

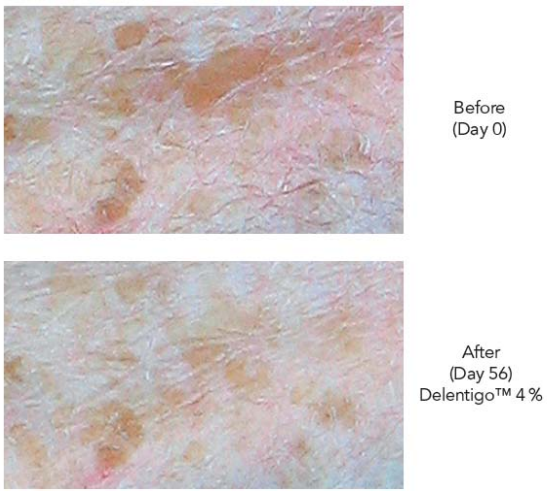

Figure 7 Photos of age spots on the hand of one subject, before and after treatment

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