THE NON-STOP SEARCH FOR EFFICIENT WHITENING

Nowadays, what every woman wants is a complexion of pure porcelain. There is an ongoing consumer trend to brighten the skin to give it a more even tone. Furthermore, skin lightening is considered to be an antiageing facet of skincare. In Asia, there is significant demand for whitening products to either lighten the skin complexion generally or to adjust variations in pigmentation. For Caucasian skin, whitening products are used to treat age spots or other forms of hyperpigmentation, such as freckles or darkly pigmented scars.

PIGMENTATION PROCESS

The pathway from exposure to UV light to pigmentation is very complex and contains many steps. UV light leads to the generation of reactive oxidants in keratinocyte cells that, in turn, release inflammatory mediators such as prostaglandins, nitric oxide (NO) and α-melanocyte-stimulating hormone (α-MSH). There are receptors for both prostaglandins and α-MSH on melanocyte cells and a lot of research has been done on a specific receptor for α-MSH called melanocortin 1 receptor (MC1R). After binding with α-MSH, the receptor induces its host melanocyte to promote the expression of the tyrosinase gene and to enhance dendricity. Tyrosinase is the rate-limiting enzyme in the synthesis of melanin pigments. Melanin is produced in specialized organelles, called melanosomes, which are gradually filled with pigments, transported to the peripheral dendrite tips and then transferred to the surrounding keratinocytes. There, melanosomes form a protective shield around the cell nucleus, producing a uniform skin colour.

In the past, unwanted pigmentation was treated by using actives that inhibited or reduced the enzymatic activity of tyrosinase. The whitening actives marketed today interfere at different stages of the pigmentation cascade. A new series of actives was introduced that reportedly blocked the transfer of melanosomes to the keratinocytes. Another efficient way to suppress pigmentation would be to block the upregulation of the expression of tyrosinase and to inhibit the stimulation of melanocyte dendricity. This could be achieved by interfering with the binding of keratinocyte mediators to their receptors on the melanocytes or by inhibiting the production of these mediators.

With age spots, two types of pigments are present, the melanins and lipofuscin. They are overproduced in the spot area because of an overreaction to UV radiation. In keratinocytes, UV light leads to the generation of free radicals and reactive oxygen species that eventually leads to the oxidation of proteins and lipids. Oxidized proteins and lipids form insoluble, darkly pigmented complexes called lipofuscin. The 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, explaining why uneven pigmentation is a typical symptom of old age.[1] The cosmetic approach to fading or hiding age spots is to use whitening products that are only applied to the spot area. Chemical peels give more obvious results but, if not solely applied to the spot area, the fading effect is minimal because the bleaching will whiten the whole facial area.

NATURALLY EFFECTIVE

Sulforaphane is a plant substance belonging to the isothiocyanate group. Isothiocyanates are sulphur-containing chemicals that are characteristic of the Brassicaceae family. Sulforaphane works as an indirect antioxidant.[2] Direct antioxidants, such as vitamins C and E, can neutralize an oxidant once and then need to be replenished by other antioxidants. Indirect antioxidants turn on the expression of a series of genes that code for cytoprotective proteins. These proteins are enzymes that synthesize or regenerate a lot of

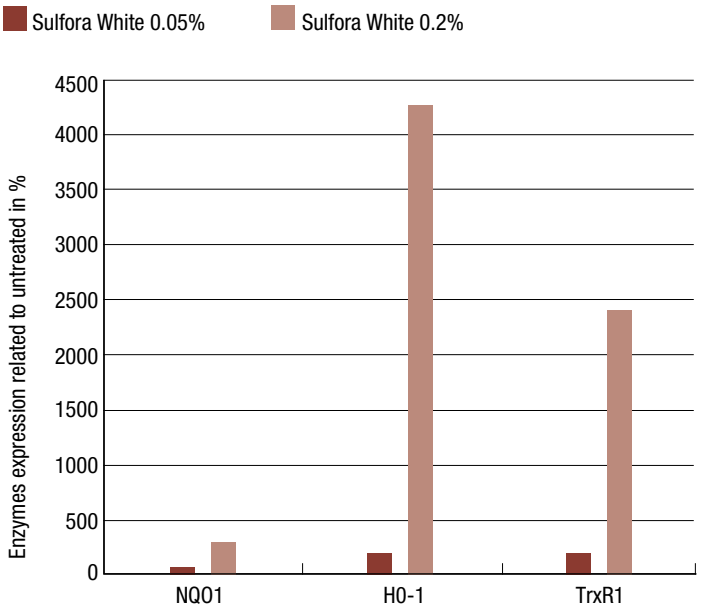

Figure 1: Effect of SulforaWhite on the expression of antioxidant enzymes. The expression of NADPH:quinone reductase 1 (NQO1), heme oxygenase 1 (HO-1) and thioredoxin reductase 1 (TrxR1) was measured using real-time PCR.

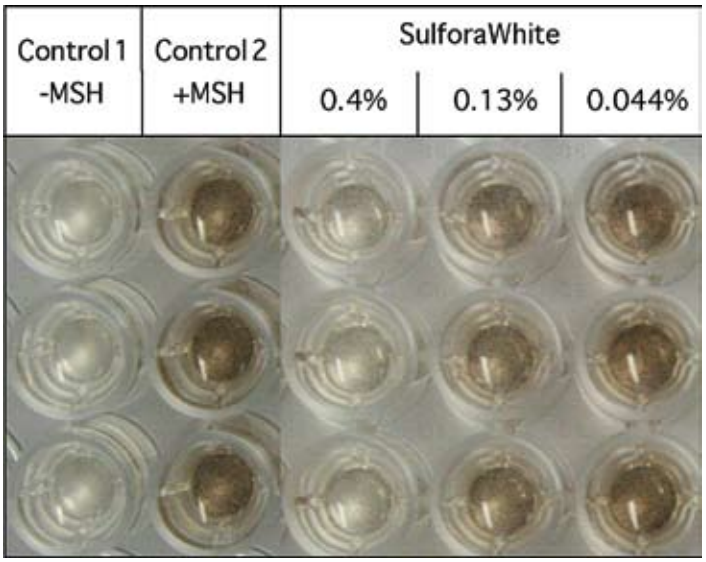

Figure 2: Inhibition of melanin production in B-16 melanocytes.

different direct antioxidants. In cress sprouts, for example, the sulforaphane concentration is 20–50 times higher than in mature vegetables. Four to five day old garden cress sprouts were used as the raw material to produce the commercially available ingredient, SulforaWhite.

To distinctively treat age spots, a cosmetic ingredient that combines an aqueous cress sprout extract and the soy isoflavone, genistein, was developed (Delentigo).

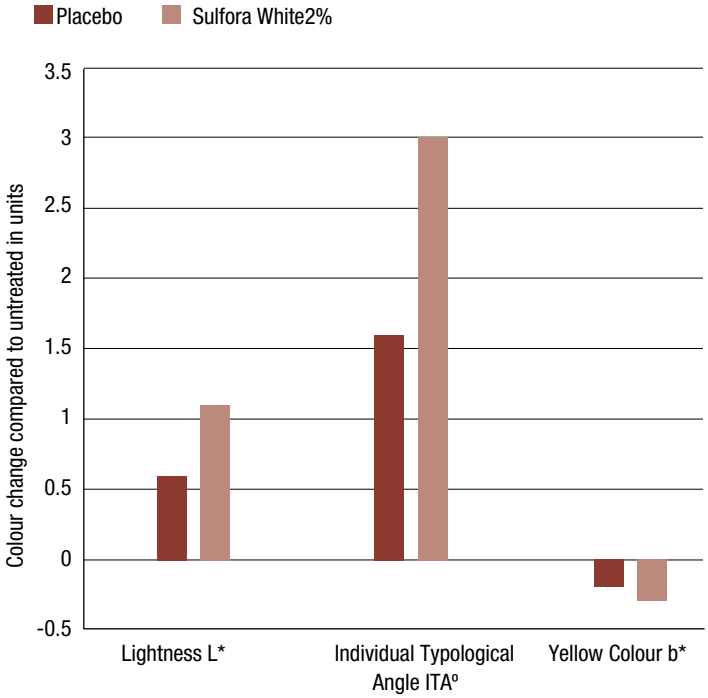

Figure 3: In vivo test results. Skin whitening was determined by chromametric measurements after treatment with 2% SulforaWhite.

Genistein is a well-known natural inhibitor of tyrosine kinase, which is reported to be located on the intracellular side of the stem cell factor (SCF) receptor.[3] There are also reports of the involvement of tyrosine kinase in ET-1 (enthothelin-1) signalling. The messenger molecules ET-1 and SCF are known to be highly overproduced in age spot areas.[4] This explains the high melanin content compared with neighbouring, normally pigmented skin. Therefore, genistein inhibits the effects of SCF and — probably — ET-1 as well on melanocytes. Delentigo activity takes place exclusively in the spot area without provoking a bleach effect on the adjacent skin.

STUDY RESULTS

Expression of antioxidant enzymes: The capacity of SulforaWhite to promote the expression of antioxidant enzymes was analysed in vitro using normal human epidermal keratinocytes. The method of real-time polymerase chain reaction (PCR) was used to measure the expression of selected genes. Several antioxidant enzymes were chosen as representatives of Phase II (carcinogen defence) enzymes.

- NADPH:quinone reductase 1 (NQO1) is a major anticarcinogenic 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 defence.
- Thioredoxin reductase 1 (TrxR1) works together with NADPH to control the redox balance of the cell.
- Glutathione peroxidase (GPX1) has a major role in the reduction of lipid peroxides and of free hydrogen peroxide.

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% SulforaWhite. After incubation, the cells were harvested and total RNA was extracted. Compared with the untreated control, the antioxidant enzyme NQO1 was moderately stimulated at 0.05% and strongly stimulated at 0.2% SulforaWhite (Figure 1). HO-1 and TrxR1 were both stimulated strongly, even at the lower SulforaWhite concentration. The enzyme GPX1 did not respond to SulforaWhite in this trial.

Inhibition of melanin production: In an in vitro study conducted on melanocytes, the garden cress sprout extract was shown to inhibit melanin formation. Specific cells (B-16 melanocytes) were cultivated with NDP-MSH, a stable derivative of the natural hormone α-MSH that stimulates skin pigmentation. The melanin content was determined by measuring its optical density at 405 nm and the cell viability was evaluated in parallel using an MTT assay. Results showed that 0.4% SulforaWhite inhibits melanin formation induced by α-MSH by 44% (Figure 2). This activity is dose-

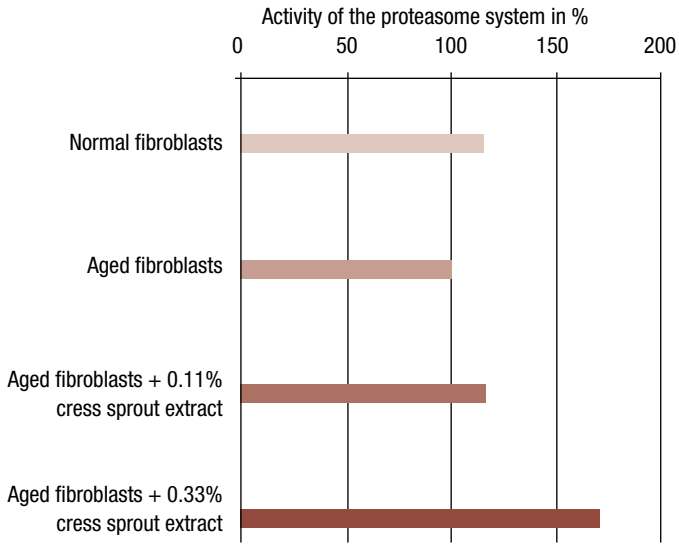

Figure 4: Determination of fibroblast proteasome activity and the effect of a cress sprout extract.

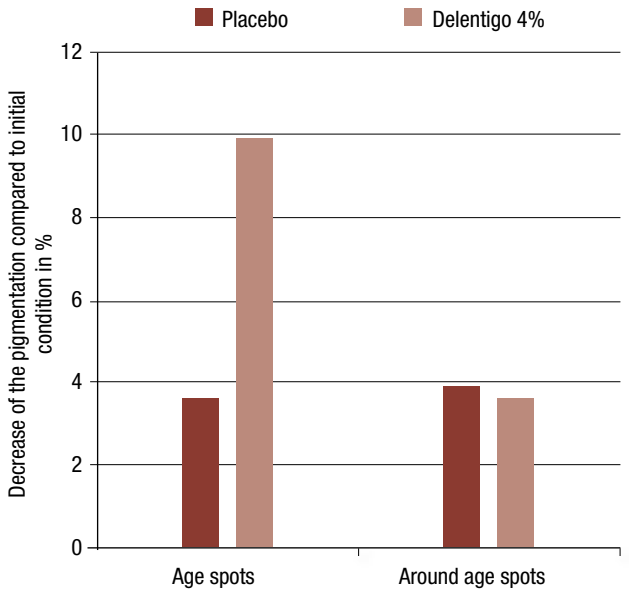

Figure 5: In vivo test results. The melanin index of age spots was measured after treatment with a 4% Delentigo cream.

dependent and is not a cytotoxic effect artifact as the MTT assay showed.

Whitening effect of SulforaWhite: A human clinical trial was conducted on 22 Asian subjects aged between 22 and 39. A cream with 2% SulforaWhite was applied twice daily for 56 days on the inner side of one forearm. The other forearm was treated with a placebo cream. The upper arm was used as an untreated zone. Skin colour was measured with a MINOLTA CR300 chromameter. Whitening is shown by increased skin clarity, measured as lightness L*, and by an increase in Individual Typological Angle (ITA°). After 56 days of use, and compared with the placebo product, the cream with SulforaWhite induced a significant increase in lightness L* (+0.5 ± 0.2 AU; $p = 0.004$) and a significant increase in the ITA° parameter (+1.4 ± 0.4 AU; $p = 0.004$) (Figure 3).

Specific age spot whitening with Delentigo: The cress sprout extract turned out to be very active in a cell-based screening assay for modulators of the proteasome system. Proteasome activity was evaluated by using the Proteasome-Glo Assay (Promega) using a substrate whose degradation by the proteasome results in a luminescent signal. Results show that the proteasome activity in young fibroblasts (passage 8) was 15% higher than in old cells (passage 16) (Figure 4). Four hours after incubation with the cress sprout extract (0.33%), the proteasome activity of old fibroblasts was increased by 71%. By stimulating proteasome activity, the cress sprout extract can prevent or reduce the accumulation of oxidized proteins and thus inhibit lipofuscin formation.

A 4% Delentigo cream was tested in two clinical studies. The first double-blind, placebo-controlled study was done with 10 women aged between 47 and 77. The test cream was applied twice daily for 4 weeks on defined spots as well as on normally pigmented skin areas of one hand. The placebo cream was applied in a similar way to the other hand. To analyse the skin pigmentation, the melanin index was measured with the Skin Pigmentation Analyzer SPA99 (Courage and Khazaka) at the beginning of the study and after 4 weeks. The study results showed that the test cream could significantly fade the age spots (Figure 5). After four weeks' application and compared with age spots treated with the placebo cream, the melanin index had decreased by 6.2%. There was no difference in the melanin index of the normally pigmented skin treated with either the test cream or the placebo.

CONCLUSIONS

A garden cress sprout extract was demonstrated to exert a significant whitening activity. The results of the cell-based assays with B16 cells indicate that SulforaWhite disrupts the binding of α-MSH to its receptor on melanocytes. The whitening mechanism of SulforaWhite also seems to be linked to its capacity to protect against reactive oxidants, the very first triggers in the pigmentation cascade. As such, SulforaWhite exerts a skin whitening effect and, at the same time, protects against premature skin ageing. Age spots are caused by a local overreaction to sunlight. The result is an increased formation of two types of pigments, namely melanin and lipofuscin. For a treatment to be efficient, the synthesis of both types of pigments has to be inhibited. Genistein, a known tyrosine kinase inhibitor, is supposed to interfere with the SCF/ET-1 cytokine signalling that takes place between keratinocytes and melanocytes (shown in the literature to be overactive in age spots). The cress sprout extract blocks the activity of the sunlight-induced paracrine factor, α-MSH. All these activities result in the reduced formation of melanin. The cress sprout extract acts as a promoter of the cell's own defence against oxidative stress and as a specific stimulator of the proteasome system — reducing the formation of lipofuscin. Therefore, Delentigo acts specifically in the spot area without inducing a bleaching effect on the neighboring skin. PHM

REFERENCES

1. V.A. Vernace, T. Schmidt-Glenewinkel and M.E. Figueiredo-Pereira, "Aging and Regulated Protein Degradation: Who Has the Upper Hand?" *Aging Cell* **6**, 599–606 (2007).
2. P. Talalay, *et al.*, "Sulforaphane Mobilizes Cellular Defenses That Protect Skin Against Damage by UV Radiation," *Proc. Natl Acad. Sci. USA* **104**, 17500–17505 (2007).
3. R. Roskoski, "Signaling by Kit Protein-Tyrosine Kinase: The Stem Cell Factor Receptor," Biochem. *Biophys. Res. Commun.* **337**, 1–13 (2005).
4. G.E. Costin and V.J. Hearing, "Human Skin Pigmentation: Melanocytes Modulate Skin Color in Response to Stress," *FASEB J.* **21**, 976–994 (2007).

FOR MORE INFORMATION

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