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mibellebiochemistry

# Mibelle Biochemistry, an expert for cosmetic actives, developed SulforaWhite, a phyto agent for visible whitening based on sulforaphane

Sulforaphane is a plant substance belonging to the isothiocyanate group. Isothiocyanates are sulphur-containing chemicals that are characteristic for 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 became famous as the health promoting active in broccoli. Its anti-cancer effects have been scientifically proven. A recent study done at the Johns Hopkins University in Baltimore showed that broccoli extract also boosts the defence system of the skin against UV rays. Sulforaphane works as an indirect antioxidant. 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 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 different direct antioxidants. Enzymes work catalytically, meaning that they are able to do thousands of working steps without being consumed. Mibelle Biochemistry used garden cress sprouts as a raw material to produce a cosmetic ingredient with the active sulforaphane (SulforaWhite). In a clinical study, a remarkable whitening effect could be demonstrated for SulforaWhite. In studies with keratinocytes, SulforaWhite was able to highly stimulate the expression of cytoprotective genes and as a consequence to protect keratinocytes against harmful free radicals. Studies with melanocytes showed that SulforaWhite prevented the binding of the alpha-melanocyte stimulating hormone (α-MSH) to its receptor on melanocytes. Thus, the whitening mechanism of SulforaWhite is a combination of blocking UV-induced free radical formation and α-MSH-signalling.

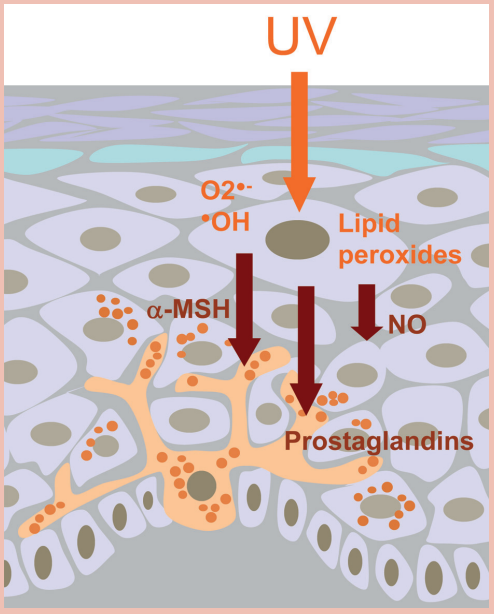


Figure 1. Signalling mediators involved in the tanning response

## GARDEN CRESS SPROUTS: A RICH SOURCE OF SULFORAPHANE

The sprouts of the Brassicaceae vegetables contain the highest concentration of isothiocyanates. In sprouts, the sulforaphane concentration is 20 to 50 times higher than in mature vegetables. Garden cress is suitable for hydroponic cultivation and is typically harvested just a week after germination. 4 to 5 day old garden cress sprouts were used as the raw material to produce SulforaWhite. The composition (INCI) is: Lepidium Sativum Sprout Extract, Glycerin, Lecithin, Phenoxyethanol and Aqua. For a better skin uptake, the actives of SulforaWhite are incorporated into liposomes.

| Concentration of SulforaWhite (%) | 0.05 | 0.2 |
|---|---|---|
| | Expression (% to untreated) | |
| **Cytoprotective enzymes** | | |
| NADPH:quinone reductase 1 | 175 | 314 |
| Heme oxygenase 1 | 312 | 4282 |
| Thioredoxin reductase 1 | 284 | 2416 |

Table 1. Effect of SulforaWhite on the expression of cytoprotective enzymes in keratinocytes

## REGULATION OF SKIN PIGMENTATION

The process from exposure to UV light to pigmentation is very complex and contains many steps. As shown in Figure 1, UV light leads to the generation of free radicals and reactive oxygen species in keratinocytes. The primary free radicals superoxide anion $O2^{\bullet-}$, the hydroxyl radical •OH

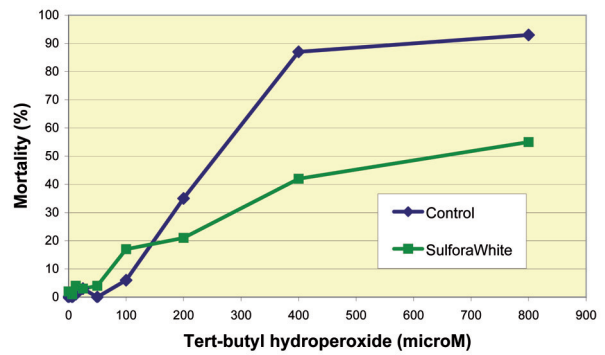


Figure 2. Protective effect of SulforaWhite in keratinocytes against tert-butyl hydroperoxide

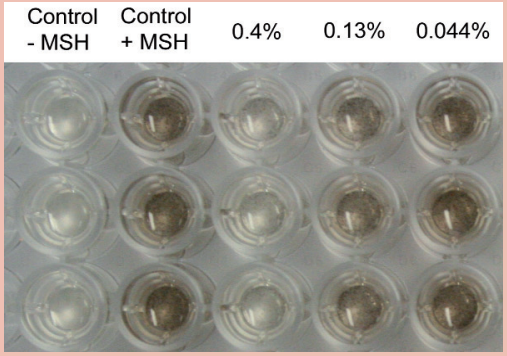


Figure 3. Concentration-dependent inhibition of melanin formation by SulforaWhite in melanocyte

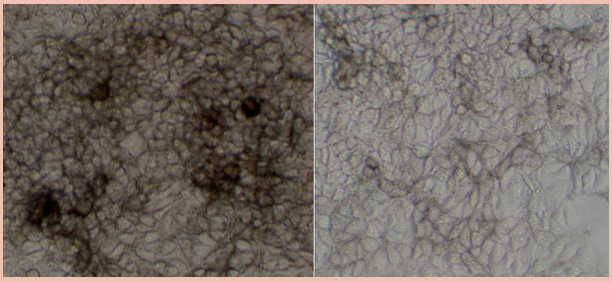

Figure 4. Microscopic photos of the B16 murine melanocyte monolayers (left: control with α-MSH; right: with 0.4% SulforaWhite)

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). There are receptors for prostaglandins and α-MSH on melanocytes. A lot of research was done on the receptor for α-MSH, called melanocortin 1 receptor (MC1R). After binding with α-MSH, the receptor induces melanocytes 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. 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 colour. In the past, pigmentation was inhibited mainly by actives that reduced the enzymatic activity of tyrosinase. The whitening actives marketed today interfere at different steps in the pigmentation cascade. A series of new actives came up that were reported to block the transfer of melanosomes to keratinocytes. Another efficient way to suppress pigmentation would be to block the upregulation of the expression of tyrosinase and to block the stimulation of melanocyte dendricity. This could be achieved by interfering with the binding of keratinocyte mediators to their receptors on melanocytes or by inhibiting the production of these mediators. Exactly these are the steps where SulforaWhite interferes with the regulation of skin pigmentation.

## THE CAPACITY OF SULFORAWHITE TO NEUTRALIZE FREE RADICALS AND REACTIVE OXYGEN SPECIES IN KERATINOCYTES

The capacity of SulforaWhite to promote 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 selected genes. Several antioxidant enzymes were chosen as representatives of cytoprotective enzymes. 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. Glutathione peroxidase (GPX1) enzyme 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 to the untreated control, the antioxidant enzyme NQO1 was moderately stimulated at 0.05% and strongly stimulated at 0.2% SulforaWhite (Table 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. SulforaWhite was found to stimulate the expression of cytoprotective enzymes. As consequence, cells in culture pretreated with SulforaWhite should be more resistant against toxic free radicals. This protective effect of SulforaWhite was analyzed in vitro using normal human epidermal keratinocytes. The chemical stressor used was tert-butyl hydroperoxide (t-BH), a strong oxidizing organic peroxide. The keratinocytes were precultured in standard growth medium. Then the cells were pre-treated for 24 hours with 0.05 % SulforaWhite. After this pre-treatment, the culture supernatant was removed and standard growth medium with t-BH was added. After 4 hours' incubation, the cell viability was measured by MTT assay. Cell viability was analyzed again 20 hours after incubation with the stressor. SulforaWhite exerted an impressive protection against t-BH (Figure 2). The IC50 value, indicating the concentration of the stressor inducing 50 % mortality, increased from 258 µM in the control without SulforaWhite to 646 µM in the culture pre-treated with SulforaWhite. The protective effect persisted even after 20 hours after incubation with t-BH.

## THE EFFICACY OF SULFORAWHITE TO INHIBIT MELANIN FORMATION IN MELANOCYTES

Inhibition of melanin formation was confirmed 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 α-MSH. After incubation, the melanin content was analyzed by measuring optical density at 405 nm. A plate that was cultivated in parallel was used for the evaluation of cell viability by the MTT assay. SulforaWhite was tested at three different concentrations. Melanin formation was strongly inhibited at 0.4%. The results are illustrated in Figure 3 and 4. The MTT assay clearly demonstrated that this was not the consequence of a cytotoxic effect. The inhibitory effect of SulforaWhite on melanin formation after stimulation with α-MSH could also be demonstrated with normal human melanocytes. SulforaWhite at 0.016% reduced melanin synthesis by 47%. SulforaWhite 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 SulforaWhite antagonises binding of α-MSH to its receptor on melanocytes.

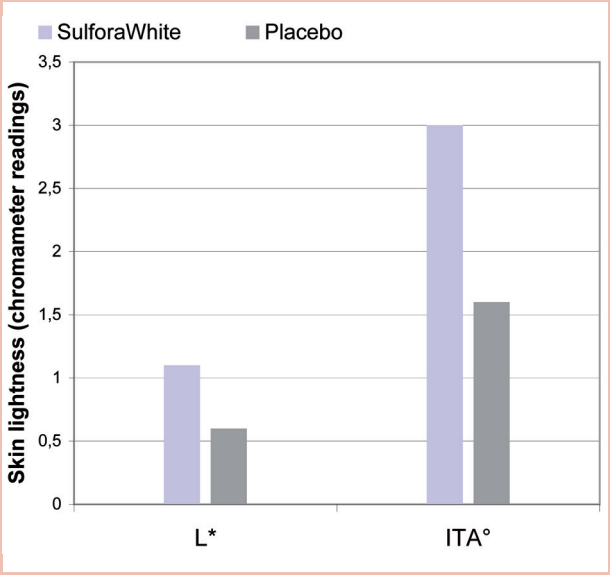


Figure 5. Chromameter results of the clinical whitening study with SulforaWhite

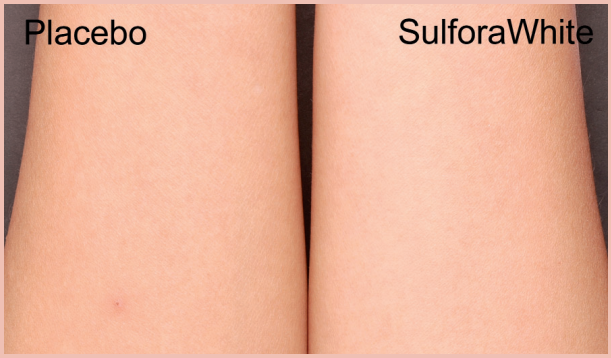


Figure 6. Photos of the inner side of the forearms of one subject

## CLINICAL STUDY DEMONSTRATES WHITENING EFFICACY 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 the placebo cream. The upper arm was used as an untreated zone. Skin colour 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 made. The chromameter results showed a clear placebo controlled whitening effect (Figure 5). After 56 days of use, and compared to the placebo product, the cream with SulforaWhite 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 6 demonstrates that the whitening effect was visible to the naked eye.

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