**TEGO Cosmo C 250 as a mild Skin Brightener**

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**Introduction**

Skin color varies depending on several influences like racial background or season of the year. The type, quantity and distribution of melanin in the epidermis plays a crucial role in the determination of skin color.

In recent years, the suppression of the melanin synthesis for cosmetic reasons to obtain a light skin became more important. In Asia skin lightening products became the best selling skin care products, whereas in the Western hemisphere including Europe and North America the main driving force for their use is the demand for age-spot treatment and evening of the skin tone.

With the cytotoxic and sensitizing side effects of established skin brighteners such as hydroquinone and kojic acid, it is apparent that there is a strong need for sophisticated active ingredients with skin lightening efficacy and safety. Additionally, stability issues leading to an unwanted, strong coloring of existing formulations are known for vitamin C derivatives, arbutin and plant extracts.

With TEGO® Cosmo C 250 a mild active ingredient for skin lightening products is provided which does not possess any cytotoxicity and besides that is easy to formulate.

TEGO® Cosmo C 250 (INCI: 1-Methylhydantoine-2-imide) is a natural amino acid derivative with skin brightening properties. It belongs to the class of guanidino compounds which are ubiquitous present in mammalian cells.

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**Melanogenesis**

Melanin pigments play a major role in photoprotection of the skin. Melanin limits the extent of UV penetration through the epidermal layers, and protects cutaneous structures and delays the occurrence of UV-induced inflammation and erythema (sunburn). In addition, these pigments are also potent endogenous antioxidants and scavenge reactive oxygen radicals, which – if not neutralized – eventually can lead to oxidative DNA damage.

The biochemical synthesis of melanin is called "melanogenesis" and is a complex, fine-tuned process, which is central to skin homeostasis and functioning, and involves signaling between melanocytes and surrounding cells. The process of melanogenesis takes place in specific membrane-limited organelles, the melanosomes, which are located in the melanocytes, a set of specialized dendritic cells, which synthesize the pigment and transfer it eventually to neighboring keratinocytes as recipient cells.

Melanin biogenesis is a complex process which goes beyond the mere provision of pigments. Many research teams around the world have gradually contributed to the extension of knowledge about the many cellular and molecular events that are involved. Tyrosinase is a key enzyme which is the critical factor that ultimately regulates melanogenesis, since it is involved in several phases of the pigmentation process. Tyrosinase is a copper-containing glycosylated enzyme responsible for the production of melanin pigments. It is found specifically in melanocytes and is present throughout the phylogenetic spectrum.

L-tyrosine, an essential aromatic amino acid, is converted via a tyrosinase-mediated pathway into L-dopaquinone via the intermediate 3,4-dihydroxy-phenylalanine (L-DOPA). Several other regulatory oxidation and polymerization mechanisms occur downstream from the L-dopaquinone intermediate, including the rearrangement of dopachrome and the oxidative polymerization of 5,6-dihydroxyindole as well as post-synthetic structural modifications of melanin, leading to a variety of natural pigments (Figure 1).

Depending on the skin type and environmental effects the melanin pigments are synthesized in varying concentrations. Epidermal melanocytes synthesize two main types of melanin: The black eumelanin and the yellow to reddish-colored pheomelanin. Levels of pheomelanin are detected in human skin regardless of race, color, and skin type. However, eumelanin is always the major constituent of epidermal melanin, and the skin color appears to be determined by the quantity of melanin produced but not by the quality. Fair-skinned people (Type I and II) have lower levels of eumelanin than dark-skinned people and suffer as a consequence from less photo protection.

Equally contributing factors are the efficient transfer of melanin from the melanocytes to the neighboring keratinocytes with distribution and degradation of the transferred melanosomes by the recipient keratinocytes. Melanocytes are isolated cells located in the epidermis, which are supported by the basal membrane containing keratinocytes. They have globular cellular bodies from which many “arms” of different length – the dendrites – are deployed. So once the melanin pigments are synthesized, the pigmented melanosomes are translocated down the dendrites and captured at the dendritic tips via a variety of cytoskeletal elements. Keratinocytes are the ultimate recipients of the melanin and therefore represent the most abundant melanin-containing cells in the skin. The melanosomes are organized in a systematic way around the nucleus of the keratinocytes and more particularly on the upper pole of the cell. This phenomenon – “capping” – covers and protects the basal membrane keratinocyte cell nuclei and finally the DNA from UV radiations.

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UV light stimulates the rate of transfer of melanin pigments from melanocytes to keratinocytes. The close relationship between both cell types suggests mechanisms of communication between them.

Active Ingredients for Skin Brightening

In the Asian-Pacific area, a lighter skin has always been a desired attribute. Cosmetics that help to clear the skin have been labeled as skin brightening products and are considered as indispensable for daily skin treatment. Particularly in combination with UV absorbers, which assist to avoid a tanning of the skin, brightening ingredients have become a successful concept for skin care products in Asia and are becoming increasingly popular among large parts of the population.

Not quite a social phenomenon in USA and Europe yet, skin brightening formulations from a cosmetic point of view in most cases are used to control the skin’s color tone and reduce the appearance of freckles and age-spots. Irregular pigmentation is commonly associated with aging skin, because the melanocytes in photo-aged skin are working beyond capacity such that their efficiency eventually declines resulting in too much pigment being produced in some areas (age spots or solar lentigines) and too little in others (causing idiopathic guttate hypomelanosis - the reverse of age spots). In addition, the total number and function of epidermal melanocytes decrease during aging.

All these factors contribute to an increasingly wide-spread use of products that finally inhibit or prevent pigmentation of the skin and because of that global demand the market of skin brightening products is increasing steadily with healthy growth rates.

Several depigmentation mechanisms have been described to explain skin-brightening efficacy. These include:

- Suppression of tyrosinase formation
- Tyrosinase inhibition.
- The key enzyme of the melanogenesis can be inhibited by alteration of its active center. Active ingredients with reported efficacy are generally water soluble and include e.g. ascorbic acid or kojic acid. A major disadvantage of many of these ingredients is their instability in formulations.
- Reduction of melanin.

Skin brightening can be stimulated via a bleaching effect which is known to trigger the denaturation and subsequent death of the melanocytes. The most popular active ingredient with bleaching efficacy is hydroquinone. However, the destruction of melanocytes causes side effects as permanent depigmentation and an increased photo sensitivity of the skin, especially during prolonged use periods. Because of these huge disadvantages, hydroquinone is banned or at least tightly regulated in most countries for cosmetic use.

TEGO® Cosmo C 250 for Skin Brightening

The frequent or long-term use of certain ingredients may, however, be responsible for many serious cutaneous side effects. Only recently it has been reported that kojic acid is suspected to produce potentially carcinogenic effects. As a consequence, most cosmetic companies in Japan have stopped using kojic acid in their products.

Hydroquinone is known to damage cells and due to that cytotoxicity its usage is banned in Japan and Europe for skin care applications regarding to safety concerns.

Other popular active ingredients for current use in skin brightening products include vitamin C derivatives, although they cause some stability problems like color changes in existing formulations. Additionally, their topical delivery is problematic and can result in a low bio-availability due to suboptimal penetration into the skin.

As an alternative, numerous skin brighteners based upon blends of botanical ingredients are marketed in Japan and Europe. European products often contain plant or herbal extracts to lighten the skin. The development of effective cosmetic skin brightening formulations with highly colored and odorous plant extracts is, however, not without its problems since in many cases only low concentrations can be incorporated.

Concerning the existing safety and stability issues of established skin brighteners, there is an apparently strong need for safe, stable and easy-to-use active ingredients for skin lightening products.

TEGO® Cosmo C 250 (INCI: 1-Methylhydantoin-2-imide) is an amino acid derivative with mild skin brightening properties (Figure 2), suggesting it can be used as a natural alternative to and as a supplement for other skin brightening and lightening actives.

Due to its natural origin it is an attractive solution for efficient skin lightening products. It can be used in applications such as skin brightening and age-spot fading products to improve the clarity of skin colour tone. The cytotoxicity of TEGO® Cosmo C 250 was examined in an MTT assay using the in vitro model system EpiDerm™ (MatTek Corporation). The test results indicated that TEGO® Cosmo C 250 is non-cytotoxic.
An in vitro assay was used to study the efficacy of TEGO® Cosmo C 250 in the suppression of tyrosinase activity, the key enzyme of melanin synthesis.

To 10 mM DOPA as a precursor of the orange-coloured dopachrome, an aqueous solution of TEGO® Cosmo C 250 in various concentrations and 4 U of tyrosinase of mushroom origin were added. The formation of dopachrome as an intermediate in the melanin synthesis was determined photometrically at 475 nm after an incubation period of 15 minutes at room temperature.

Figure 4 shows the results of the quantitative melanin assay. On day 17 TEGO® Cosmo C 250 turns out to be as effective as kojic dipalmitate whereas it is even more effective on day 21.

The results could be confirmed by histological analysis, since no visible network of melanin was observed after the application of TEGO® Cosmo C 250 (Figure 5).

Figure 3 shows that TEGO® Cosmo C 250 is able to reduce the formation of dopachrome in a dose dependent response. It inhibits 30% of tyrosinase activity at a concentration of 135 mM (1.5%). The study shows that TEGO® Cosmo C 250 works as a moderate inhibitor of tyrosinase.

The depigmentating effect of TEGO® Cosmo C 250 at cellular level was tested in an in vitro model system. The artificial skin model MelanoDerm™ (MatTek Corp.) consists of normal, human-derived epidermal keratinocytes (NHEK) and melanocytes (NHM). The melanocytes undergo spontaneous melanogenesis under these co-cultured conditions. The cells used in the MelanoDerm™ model were of Asian origin.

TEGO® Cosmo C 250 and kojic dipalmitate as a positive standard were dissolved. Due to its cytotoxicity kojic acid was not used in its free form but as the dipalmityl ester. A defined amount of the test compounds (25 mg/cm²) were applied to the equilibrated MelanoDerm™ tissue on day 0 and re-applied every other day (on day 2, 4, 6, 8, ..., 18 and 20). The culture medium was also removed and replaced during these days.

On days 10, 14, 17 and 21 cultures were examined for morphological changes. Staining and subsequent analysis was carried out by MatTek.

Two cultures per treatment were removed for quantitative melanin assay on days 17 and 21. Therefore, tissues were homogenized in pairs and melanin was quantified after extraction with chloroform/methanol via photometric determination at 405 nm.

The efficacy of TEGO® Cosmo C 250 was compared to kojic dipalmitate as a positive control.

Under the conditions of this study, TEGO® Cosmo C 250 is capable of suppressing the melanin content in melanocytes. The histological analysis of the MelanoDerm™ tissue (Figure 5) visualized the melanin distribution. The lack of a dendritic network after the application of TEGO® Cosmo C 250 indicates that a potential mechanism is an inhibition of the melanin transfer from melanocytes to keratinocytes.

To substantiate the efficacy of TEGO® Cosmo C 250 further, an "in vivo"-screening study was performed. Within the scope of this study, four volunteers (age: 18-65 years) with "type III"-skin (Brownish skin with spots) applied a placebo cream and formulations containing TEGO® Cosmo C 250, or kojic acid (as a positive standard), over a period of four weeks to their arms.

3x3 cm² areas of skin on each subject right and left upper forearms were marked with indelible ink, baseline visual assessments and Chroma Meter measurements were performed and then the products were applied at a rate of 2 µL/cm² to the marked skin sites of the arms. Each study product was applied to the skin site via a micro-pipette and rubbed evenly over the test area using a gloved finger for 20 seconds, one site on each arm remained untreated.

Figure 5: Photographs of MelanoDerm™ inserts after treatment with skin brighteners
The testing methodology involved daily applications of the TEGO® Cosmo C 250 containing emulsion and the positive and negative control product. Visual assessments and measurements of skin colour were recorded, using the Minolta Chroma Meter, at baseline and at week 4.

Figure 6 demonstrates the increase of skin lightness relative to the placebo formulation. The brightening efficacy was evaluated with the “L*”-parameter (luminosity) by chromatology. The graph points out the skin brightening efficacy of TEGO® Cosmo C 250.

In summary, TEGO® Cosmo C 250 shows good results in skin brightening applications, even when the mechanism of efficacy is not fully elucidated until now. Its main skin brightening effect is believed to originate from the inhibition of melanosome transfer from melanocytes into the keratinocytes.

Formulation Advantages of TEGO® Cosmo C 250

Due to its excellent water solubility, TEGO® Cosmo C 250 can be incorporated in formulations easily with recommended usage concentration between 0.1-1.5%. The obtained emulsions are stable. No changes of color could be observed like, e.g., in plant extracts.

TEGO® Cosmo C 250 can be incorporated in formulations both in its solid form or as a stock solution in already prepared cosmetic formulations (Figure 7). The incorporation of TEGO® Cosmo C 250 in cold processed formulations is possible as well.

Since any effective skin brightening ingredients exert their efficacy at the level of epidermis in the melanocytes, the combination of TEGO® Cosmo C 250 with penetration enhancers such as polyols is highly recommended.

Additionally the combination of TEGO® Cosmo C 250 with other skin brightening ingredients, which interfere with other stages in the melanin formation process such as tyrosinase inhibitors including ascorbic acid and its derivatives is suggested. Here, besides a possible reduction of potential side effects synergetic effects may be obtained because of different penetration properties and mechanisms of depigmentation.

Conclusion

As a result of its natural origin, TEGO® Cosmo C 250 represents an attractive alternative and an effective supplementation to established skin lighteners.

TEGO® Cosmo C 250 can be used in applications such as skin brightening and age-spot fading products to improve the clarity of the skin tone and is valuable to circumvent restrictions caused by regulatory issues. It functions dually as an inhibitor of the key enzyme tyrosinase and potentially interferes with the transfer of melanin to keratinocytes. It can be easily incorporated into cosmetic formulations with proven stability. Since it is an active ingredient of natural origin which occurs in all mammalian cells, it can be classified as a safe, non-cytotoxic and effective skin brightener.

References

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