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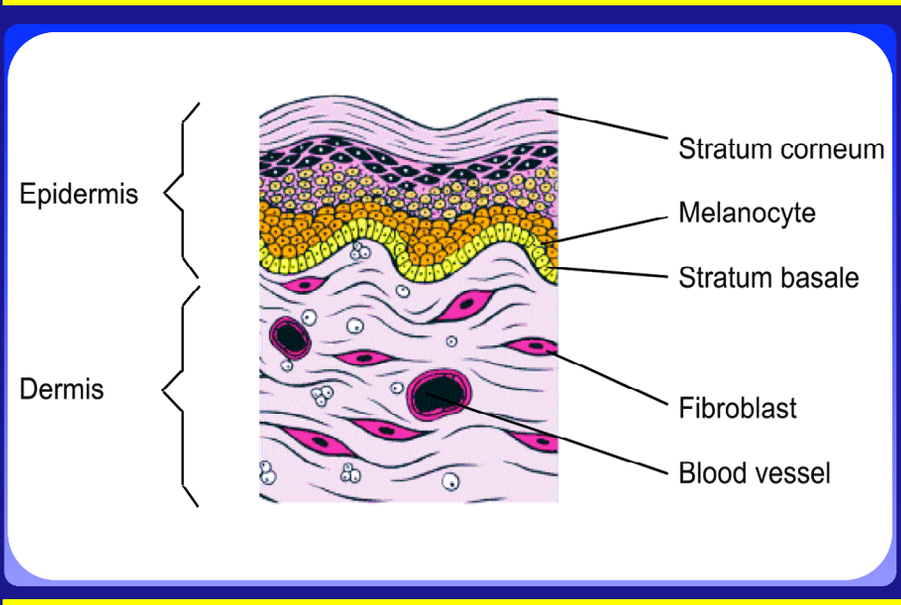

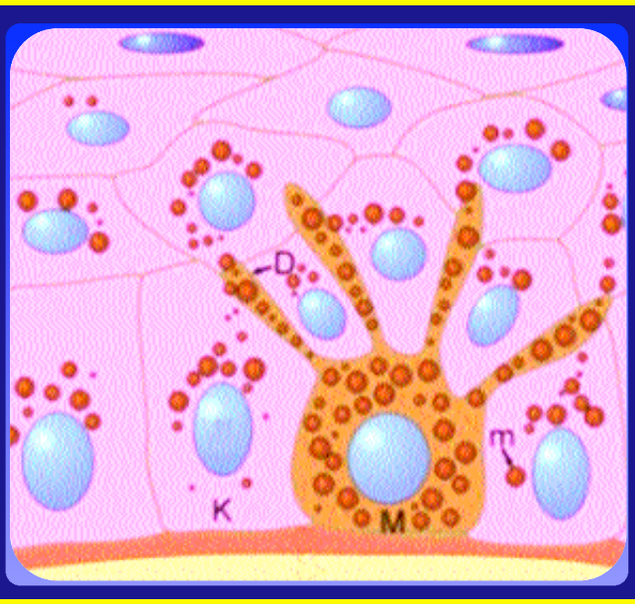

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Stimulation of Melanin Synthesis for Tanning and Protection

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Stimulation of Melanin Synthesis for Tanning and Protection

Keywords: melanin, monk's pepper, beta-endorphin, acetyl tyrosine

Abstract

The neuropeptide beta-endorphin, principally known to induce an analgesic effect and a feeling of euphoria in the central nerve system, was shown in the last years by several research groups to play also a role in peripheral organs such as the skin. There, beta-endorphin was found to participate in the regulation of pigmentation. Monk's pepper (*Vitex agnus castus*) is a shrub native to the Mediterranean area with black berries that are used as herbal medicine. Compounds of these berries were found to exert a beta-endorphin-like activity. In studies with melanocytes we could show that an extract of Monk's pepper berries stimulated specifically the melanin synthesis. *In vivo* a combination of acetyl tyrosine, a stable substrate for melanin synthesis and the Monk's pepper berries extract induced skin tanning. Detailed analysis of the in *vivo* experiment showed the individual effects of acetyl tyrosine and the plant extract.

Introduction

Pigmentation determines skin color and is the body's own protection against solar ultraviolet (UV) radiation. It consists of melanin which is a composition of mainly two substances, the black-brown eumelanin and the reddish-yellow pheomelanin. In melanocytes melanin is synthesized and stored in organelles called melanosomes. Melanocytes are located in the basal layer of the skin epidermis, in the so-called stratum basale **(Fig. 1)**. Thanks to dendrites melanocytes are able to spread melanosomes and with them the enclosed melanin within the skin by transferring them to keratinocytes **(Fig. 2)**.

The biosynthesis of eumelanin and pheomelanin can be initiated from either the hydroxylation of phenylalanine to tyrosine or directly from tyrosine. Tyrosine is hydroxylated to dihydroxyphenylalanine (DOPA) and to DOPA quinone by tyrosinase. After the generation of DOPA quinone two separated pathways which both include several intermediate steps lead to the formation of eumelanin and pheomelanin **(Fig. 3)**.

Pigmentation of the skin is controlled by hormones which are synthesized and distributed by the pituitary gland. The alpha-melanocyte stimulating hormone (alpha-MSH) is a cleavage product of the large precursor protein proopiomelanocortin (POMC) **(Fig. 4)**. It mainly regulates the pigmentation process in the skin and exerts its effect through MC1R, a G-protein coupled receptor. The gene expressions of both POMC and MC1R are inducible by UV radiation. Similar to alpha-MSH, beta-endorphin is a cleavage product of POMC in melanocytes as well **(Fig. 4)**. A research group demonstrated that the receptor for beta-endorphin, mainly the mu-opiate receptor, is also expressed in melanocytes. They concluded that the beta-endorphin/mu-opiate

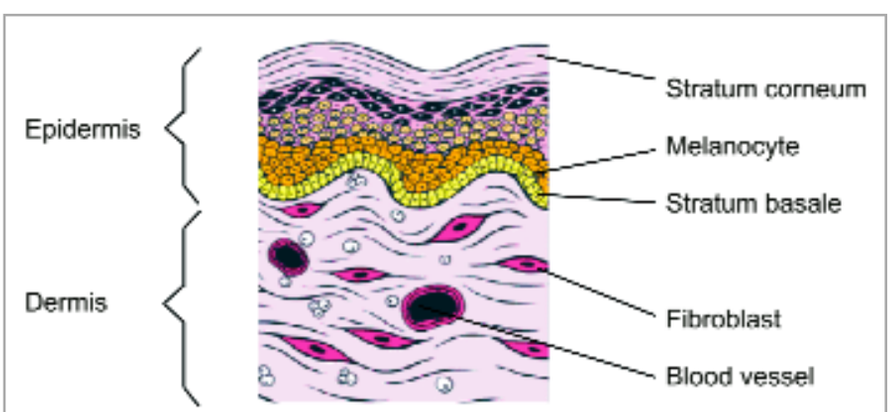

Fig. 1 Histology of the skin. The stratum basale where melanocytes are located is highlighted in yellow

receptor system is functionally active in the regulation of melanocyte biology. In melanocyte cultures beta-endorphin was found to stimulate melanocyte dendricity, proliferation, and pigmentation (1).

Tanning ingredients

In self tanning (sunless tanning) products the substance dihydroxyacetone (DHA) is often applied as a standard ingredient. It binds to proteins of the stratum corneum and stains the upper layer of skin cells. The tan results from the so-called Maillard reaction between aldehydes or ketones and the amino acid lysine, leading to a colored product. Unfortunately, such pigments do not absorb UV radiation and consequently do not provide any sun protection. In addition, the tanned outer cells peel off in the natural regeneration cycle and the skin turns pale again after a short time.
Many tanning products that stimulate the synthesis of natural melanin in the skin contain acetyl tyrosine. This substance is a natural amino acid bound to acetic acid and provides the substrate for the generation of melanin synthesized along the lines of the physiologic pathways as described in the introduction. Contrary to DHA the skin tan gained with acetyl tyrosine consists of melanin and therefore has natural sun protection qualities and remains in the skin for a much longer time.

Monk's pepper and skin tanning

Monk's pepper (chaste tree, *Vitex agnus-castus*, Verbenaceae) is a deciduous shrub domiciled in Mediterranean Europe and Central Asia. The berries contain essential oils, fatty oils, diterpenoids, ketosteroids, iridoid glycosides (agnuside and aucubine), saponins, and flavonoids. In ancient times Monk's pepper was used for several indications such as inflammation, dropsy, spleen enlargement, premenstrual syndrome, or for the treatment of injuries. In monasteries' kitchens Monk's pepper was often used as surrogate pepper taking advantage of the anaphrodisiac effect. Today, the Com-

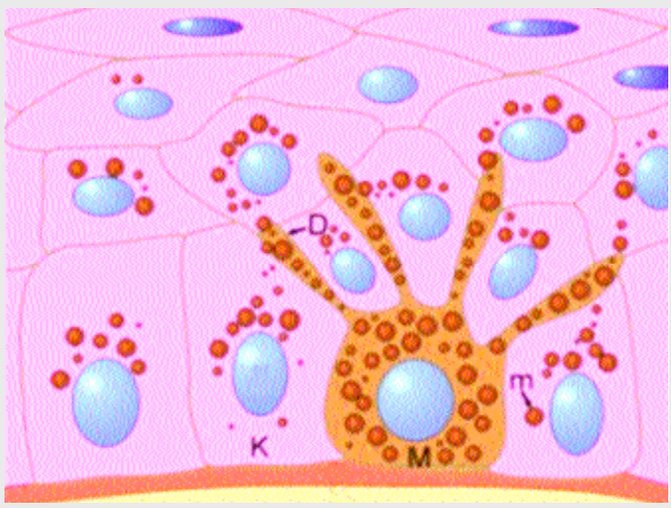

Fig. 2 Melanosomes are distributed among keratinocytes and gather on the apical side of the nucleus.
Melanocyte (M), keratinocyte (K), dendrite (D), melanosome (m)

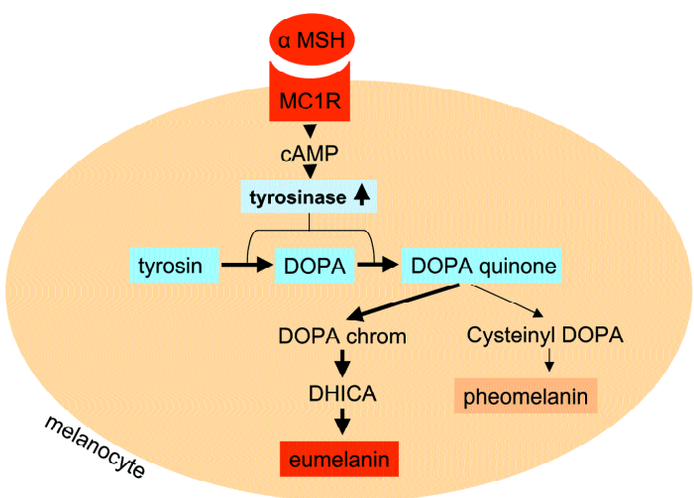

Fig. 3 The synthesis of eumelanin is stimulated by alpha-MSH through its receptor MC1R. During high tyrosinase activity the equilibrium is shifted to eumelanin synthesis

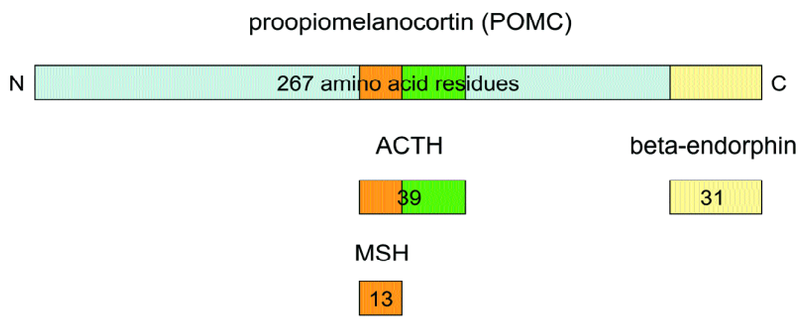

Fig. 4 Proopiomelanocortin and its cleavage products ACTH, MSH, and beta-endorphin

mission E monograph has approved the use of Monk's pepper for irregularities of the menstrual cycle, premenstrual disturbances, and mastodynia.
Monk's pepper was found in latest research to contain phyto-endorphins. This has been shown by assessing the affinity of an extract of Monk's pepper to the mu-opiate and kappa-opiate receptors. In competition binding studies with radioligands the endorphin-like compounds were observed in the lipophilic fractions of the extracts. The researchers accordingly suggested possible pharmacological effects of Monk's pepper via opioid receptors (2). Functional activity through mu-opiate receptors of the Monk's pepper extract was tested by a US research group, too. The functionality even turned out to be highly significant. Therewith, an agonistic activity of Monk's pepper extract has been demonstrated for the first time (3). Since beta-endorphins stimulate melanocytes, substances possessing agonistic functional activity through mu-opiate receptors presumably effect a similar stimulation. Monk's pepper therefore is very likely to exhibit melanogenic and dendritogenic properties like beta-endorphin as well.

MelanoBronze – combination of acetyl tyrosine and Monk's pepper extract to stimulate melanin synthesis

In MelanoBronze acetyl tyrosine and the extract of Monk's pepper cause increased formation of melanin through two different mechanisms. Acetyl tyrosine, the stable and water-soluble substrate for tyrosinase accelerates melanin synthesis. The more substrate is present, the higher the turnover of the synthesis reaction. Like UV radiation and the hormone alpha-MSH the beta-endorphin-like compounds in the extract of monk's pepper induce melanocytes to start melanin formation. In presence of the beta-endorphin activity of the extract the melanin formation is induced even without sun exposure. The individual contribution to pigmentation of acetyl tyrosine and the plant extract in MelanoBronce in presence or without sun light is shown in **Fig. 5**.

Studies and Conclusions

The effect of Monk's pepper extract on melanin production of melanocytes in culture

Melanin production and cell viability of normal human melanocytes (R6-NHEM-2) were analyzed after incubation with different concentrations of Monk's pepper extract during 10 days. Cells were cultured in a standard medium at 37 °C and 5% CO_2. After incubation melanin was extracted with a solution of 0.5 M NaOH. The optical density was measured at 405 nm against melanin standards. Cell viability was analyzed by a standard MTT assay.
Melanin production of melanocytes in culture medium alone amounted to 37.1 microg/ml. Monk's pepper extract was added in the concentrations of 0.25%, 0.13%, and 0.06%. Addition of the extract let to a concentration-dependent increased melanin synthesis of 54.5, 41.6 and 36.2 microg/ml, respectively **(Fig. 6)**. Monk's pepper extract at 0.25% in-

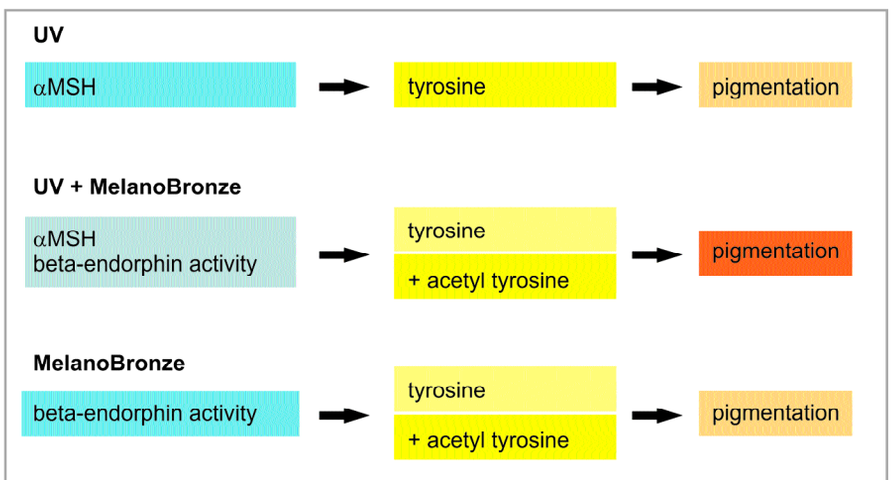

Fig. 5 MelanoBronze stimulates tanning even without sun exposure. When we are exposed to sun light, the hormone alpha-MSH is produced that induces pigmentation (upper line). When at the same time MelanoBronze is applied, pigmentation is highly accelerated because there is with beta-endorphin another inducer present and with acetyl tyrosine additional substrate for the enzyme tyrosinase (middle line). When MelanoBronze is applied, there is also tanning without exposure to sun light because MelanoBronze contains the inducer beta-endorphin and adds additional substrate for tyrosinase (lower line)

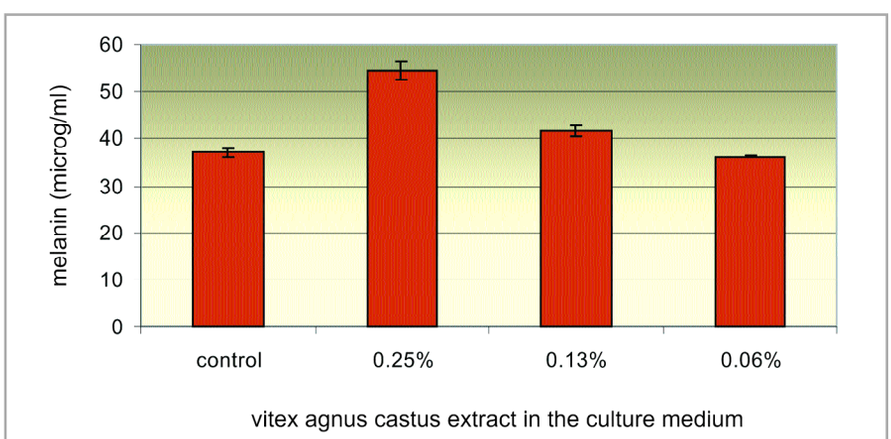

Fig. 6 The effect of Monk's pepper extract on melanin production of melanocytes in culture

creased melanin content by 47% and by 12% at 0.13%. Results of the MTT assay showed that cell viability did not change in presence of the extract compared to the control. The experiment therefore clearly demonstrated that the extract specifically induced melanin production.

The effect of MelanoBronze on skin pigmentation

Creams containing 2 and 5% of Melano Bronze and only acetyl tyrosine in a concentration corresponding to the amount of 5% MelanoBronze were compared with a placebo test cream. The test products were applied twice daily on the inner side of the forearm by 20 women with the age between 20 and 55 years. Skin tanning was initiated with UV irradiation of 1.1 MED once daily during one week. Tanning was measured with a chromameter.

After applying the placebo control cream tanning reached after one week a value of 1.83 **(Fig. 7)**. Using the creams containing 2% and 5% MelanoBronze tanning could be clearly enhanced to values of 2.15 and 2.72, respectively. Tanning with the cream containing only acetyl tyrosine reached 2.3.

The study showed that application of creams with MelanoBronze resulted in a significant and dose dependent enhanced skin tanning compared to the placebo cream. Comparing the effects of 5% MelanoBronze with the same concentration of acetyl tyrosine alone clearly demonstrated the distinct *in vivo* tanning efficacy of the Monk's pepper extract.

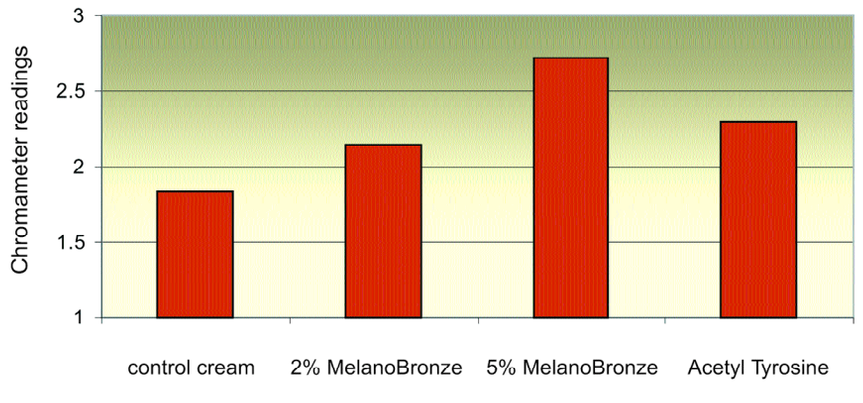

Fig. 7 Stimulation of tanning by MelanoBronze. The last column shows the tanning effect of a product with only acetyl tyrosine, in the same concentration as present in the 5% MelanoBronze test product

Summary

MelanoBronze stimulates melanin synthesis by two different mechanisms. The beta-endorphin-like effect of Monk's pepper leads to induction of the melanin formation process, independent of sun exposure, whereas acetyl tyrosine accelerates the melanin synthesis reaction. The contribution of both factors in stimulation of tanning could be demonstrated *in vivo*.

Stimulation of melanin synthesis leads to a regular, even, and persistent skin tan. In contrast to self tanning ingredients based on aldehydes or ketones Melano Bronze enhances the natural protection factor of the skin against UV radiation. For Melano Bronce there are many different applications possible. The benefits of Melano Bronze in skin care products are a fine, healthy tan and additional protection against UV radiation, the principal cause of skin aging. Thanks to its tanning and protective effects MelanoBronze is highly suitable for sun protection products as well. It is furthermore perfect for application together with self tanning ingredients like DHA and for tanning bed lotions to accelerate the tanning process.

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