Biology of melanogenesis and the search for hypopigmenting agents

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ABSTRACT

Increased production and accumulation of melanin are characteristics of a large number of skin diseases, including melasma, post-inflammatory hyperpigmentation and lentigo. A number of clinical agents can reduce normal or abnormal pigmentation, but none of these have achieved satisfactory effects. This review discusses the mechanisms behind the different approaches. Tyrosinase is a pivotal enzyme in melanin synthesis. The majority of whitening or lightening agents act by specifically reducing the activity of tyrosinase via several mechanisms: (1) prior to melanin synthesis (interfering with its transcription and/or glycosylation); (2) during melanin synthesis (tyrosinase inhibition, peroxidase inhibition and reduction of byproducts); and (3) after melanin synthesis (tyrosinase degradation, inhibition of melanosome transfer, acceleration of skin turnover). Additional melanogenesis-associated mechanisms are also discussed.

KEYWORDS

Mechanism
Melanin
Tyrosinase

Introduction

Melanin performs a crucial role in protecting skin against ultraviolet light. It also determines skin color and several aspects of phenotypic appearance. However, abnormal melanin accumulation can potentially become an aesthetic problem. Thus, whitening or lightening cosmetics are utilized extensively for the prevention and treatment of irregular hyperpigmentation. Compounds with hypopigmenting activity are frequently used in the fields of dermatology and cosmetics for these purposes.

In this review, we deal principally with agents that can affect the activity or amount of tyrosinase. We also discuss additional agents with different mechanisms of action.

Regulation of tyrosinase and related enzymes

Inhibition of tyrosinase activity

The majority of hypopigmenting agents are tyrosinase inhibitors, although their classification is complicated by their different mechanisms. Some representative examples are discussed below.

Hydroquinone is a well-known tyrosinase inhibitor. The oxidation products of hydroquinone are thought to cause oxidative damage to membrane lipids and proteins including tyrosinase, as well as glutathione depletion.1 Thus, the use of hydroquinone in cosmetics has been banned by the European Committee, because of possible long-term complications.2 Several other phenolic compounds have been identified as depigmenting agents, and act as possible alternative substrates for tyrosinase. The presence of a hydroxyl group and an electron donor group has been suggested to be a fundamental requirement for this activity.3

Compared with hydroquinone, arbutin, a naturally occurring hydroquinone beta-D-glucopyranoside, is commonly utilized in the production of cosmetics, as it is known to exhibit hypopigmenting activity at non-toxic concentrations.4 Arbutin has been demonstrated to attenuate tyrosinase activity without affecting its mRNA expression, and inhibits 5,6-dihydroxyindole-2-carboxylic acid polymerase activity.5

Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one) is an antibiotic generated by many species of Aspergillus and Penicillium. Its hypopigmenting activity has been attributed
to its chelating ability and its nuclear factor-kappa B (NF-kB) activation-inhibitory effects in keratinocytes.\textsuperscript{6,7}

Gentisic acid and its methyl ester (2,5-dihydroxybenzoic acid), a natural product of Gentiana root, inhibit melanin synthesis in melanocytes at non-cytotoxic concentrations.\textsuperscript{8}

We also screened a range of tyrosinase inhibitors. The effects of 4-n-butylresorcinol on melanogenesis were evaluated in a spontaneously immortalized mouse melanocyte cell line, Mel-Ab, where it was shown to effectively inhibit tyrosinase activity in a cell-free system, and appeared to function as an effective direct tyrosinase inhibitor (Figure 1\textsuperscript{9}). In contrast, p-coumaric acid did not directly inhibit tyrosinase activity, although it was able to induce hypopigmentation in cells. Competitive inhibition was demonstrated between p-coumaric acid and tyrosine. This may indicate that an alternative substrate of tyrosine can be used to induce hypopigmenting effects.\textsuperscript{10}

**Decreased tyrosinase production**

The transcription of genes encoding tyrosinase and tyrosinase-related protein-1 is under the control of the microphthalmia transcription factor (MITF).\textsuperscript{11} MITF belongs to the basic helix-loop-helix-zip family of transcription factors, and is crucial for both melanocyte proliferation and melanogenesis. Mutations in human MITF result in hypopigmentation and deafness in type 2A Waardenburg syndrome. As MITF is regulated by the Wnt signaling pathway, the p38 signaling pathway and the MAP kinase pathway as well as by cAMP, any agents that regulate these signaling pathways also have the potential to affect MITF and melanogenesis.

We have been searching for a signaling regulator that might also function as a hypopigmenting agent. For example, sustained extracellular signal-regulated kinase (ERK) activation by sphingosine-1-phosphate (S1P) can result in MITF phosphorylation and degradation, which are in turn responsible for reduced melanin synthesis (Figure 2).\textsuperscript{12} Transforming growth factor (TGF)-β1 also plays an inhibitory role in pigment formation. We found that TGF-β1 induced a significant delay in ERK activation and ERK-induced MITF downregulation, which could contribute to hypopigmentation.\textsuperscript{13} We also found that lysophosphatidic acid and C2 ceramides were capable of inducing MITF degradation or blocking MITF expression, respectively, mediated by an initial effect on AKT/protein kinase B or ERK.\textsuperscript{14-16} Additional evidence regarding the involvement of the ERK pathway in melanogenesis was recently uncovered, using another signaling-lipid mediator, sphingosylphosphorylcholine.\textsuperscript{17} This agent may inhibit melanogenesis via transcriptional regulation of the tyrosinase gene.
Combining melanogenesis and hypopigmenting agents

Several studies have reported on the hypopigmenting effects of fatty acids on melanogenesis. These effects are quite complex, as unsaturated linoleic acids reduce tyrosinase activity, while saturated palmitic or stearic acids increase it. Topical application of linolenic, linoleic and oleic acids (in decreasing order of efficiency) produced a bleaching effect on guinea pig skin after stimulation with UV light. The number of melanosomes and the level of tyrosinase mRNA did not appear to be influenced, suggesting that the hypopigmenting effects associated with linoleic acid were probably attributable to a reduction in the amount of tyrosinase due to the stimulation of tyrosinase ubiquitination and proteasomal degradation. As an unsaturated fatty acid, phospholipase D2 also reduces melanogenesis via the same mechanism, i.e. ubiquitin-mediated degradation of tyrosinase. The above compounds provide examples of agents that suppress melanogenesis via the increased degradation of tyrosinase proteins.

**Increased tyrosinase degradation**

Terrein, a bioactive fungal metabolite isolated from a Penicillium species, has recently been reported to reduce melanin synthesis by reducing tyrosinase production via ERK activation, followed by the downregulation of MITF. Interestingly, terrein also reduced melanogenesis via ubiquitin-dependent proteasomal degradation, as well as via decreased expression of its mRNA. Terrein is therefore an example of a hypopigmenting agent that inhibits melanogenesis by dual actions, including the downregulation of transcription and the upregulation of degradation (Figure 3).

In contrast to multifunctional terrein, the combined use of two agents with different mechanisms of action can produce additive effects. As described above, 4-n-butylresorcinol failed to induce ERK or Akt activation, or MITF degradation. It also had no effect on cAMP response-element binding protein phosphorylation, which stimulates MITF expression. However, 4-n-butylresorcinol showed an additive effect when combined with hinokitiol, which reduces MITF expression. Thus, the combination of two agents with different mechanisms may be another useful strategy for increasing the efficacy of these agents.

**Modification of tyrosinase proteins**

Tyrosinase is a copper-containing transmembrane glycoprotein and the rate-limiting enzyme in mammalian melanogenesis. Mutations at glycosylation sites have thus been implicated in oculocutaneous albinism in humans, characterized by inactive tyrosinase and the total absence of pigmentation. Glucosamine or tunicamycin, which are specific inhibitors of lipid carrier-dependent glycosylation, have been shown to induce marked hypopigmentation, coupled with ultrastructural alterations. Additionally, calcium D-pantetheine-S-sulfonate, probably generated via the alteration of tyrosinase and TRP-1 glycosylation, exerts an inhibitory effect on melanogenic enzymes, without affecting their expression, and causes reversible hypopigmentation in normal human melanocytes.

**Regulation of melanosome formation**

Melanosomes are specialized subcellular organelles in which melanin is synthesized and deposited. Electron microscopic, cytochemical, genetic and biochemical evidence all support the notion that melanosomes are specialized lysosomes. The relationship between melanosomes and lysosomes has important implications for the chemistry of melanization and the potential pharmacologic manipulation of the process. Thus, melanosome formation is a crucial step in the process of melanization.

**Interference with melanosome maturation**

The addition of TGF-β1 to melanocytes yielded significantly more stage III melanosomes, even when the cells were treated concomitantly with α-melanocyte-stimulating hormone to increase the production of fully melanized stage IV melanosomes. It has also been reported that sustained ERK activation by S1P can result in hypopigmentation.
Interestingly, a decreased number of pigmented melanosomes was detected in S1P-treated melanocytes (unpublished data). Moreover, S1P-treated cells produced undifferentiated early-stage melanosomes, whereas the control cells produced melanosomes with internal fibrils and dense pigmentation. These findings suggest that skin pigmentation might represent another strategy for regulating melanosome formation. However, no currently available agents use this mechanism to achieve hypopigmentation.

**Peroxidase inhibitors**

The involvement of peroxidase in the polymerization of melanogenic intermediates is suggested by the high degree of efficiency of peroxidase in the oxidation of 5,6-dihydroxyindole, a process that generates hydrogen peroxide (H$_2$O$_2$) as a by-product. The inhibition of peroxidase has been shown to cause hypopigmentation by reducing the polymerization rate of eumelanin in different experimental models. Methimazole is an antithyroid agent belonging to the thionamide group, which inhibits both mushroom tyrosinase and peroxidase. This agent induces a mild to moderate inhibition of melanization, and causes morphologic changes in melanocytes in animal models.

**Interference with melanosome transfer**

Melanosome formation is a crucial step in the melanization process, but the melanosomes must also be transferred from melanocytes to keratinocytes. A decrease in melanosome transfer can therefore induce a skin-lightening effect. The inhibition of serine protease has been shown to result in impaired activation of protease-activated receptor 2 in keratinocytes, resulting in the accumulation of melanosomes within melanocytes. Inhibition of this receptor therefore blocks melanosome transfer between these cells, thus also blocking the dispersion of pigment to keratinocytes. This suggests a potentially novel mechanism for the regulation of pigmentation, mediated by the inhibition of the keratinocyte receptor, protease-activated receptor 2. However, a previously tested unique inhibitor (RWJ-50353) has not been well characterized as a hypopigmenting agent. Additionally, centaureidine, a flavonoid glucoside isolated from yarrow, reduces dendritic growth and the transfer of melanosomes to keratinocytes. Moreover, niacinamide (vitamin B3) was also shown to inhibit melanosome transfer to keratinocytes, both in vitro and in vivo. However, the mechanism underlying this activity remains to be elucidated.

It has also been demonstrated in an in vitro model that glycosylated residues on melanocyte and keratinocyte membranes are critical for receptor-mediated endocytosis and thus for facilitating melanosome transfer. Lectins and neoglycoproteins have also been shown to inhibit melanosome transfer.

**Other mechanisms**

**Diverse antioxidants and various mechanisms**

Compounds with antioxidant properties exert hypopigmenting effects by interacting with copper at the active site of tyrosinase, or with o-quinones, thus avoiding the oxidative polymerization of melanin intermediates. Moreover, antioxidant agents inhibit the signaling process, enabling the stimulation of epidermal melanogenesis by scavenging reactive oxygen species generated in the skin after UV exposure.

Ascorbic acid can also interfere with the different steps of melanization by interacting with copper ions at the active site of tyrosinase, reducing dopaquinone, and by blocking the oxidation of 5,6-dihydroxyindole-2-carboxylic acid. α-Tocopherol (α-Toc) derivatives inhibit tyrosinase and melanogenesis in epidermal melanocytes. The antioxidant properties of α-Toc, which interfere with the lipid peroxidation of membranes and increases intracellular glutathione content, might explain its hypopigmentation effect. An alternative compound is α-Toc ferulate (α-Toc-F), a derivative of α-Toc stabilized by linkage by an ester bond to ferulic acid. α-Toc-F also inhibits melanin synthesis in normal human melanocytes, without interfering with the tyrosinase pathway.

6-Hydroxy-3,4-dihydrocoumarins are another set of novel antioxidants with α-Toc-like chemical structures that have recently been reported to exert anti-melanogenic effects in cultured normal human melanocytes at non-cytotoxic concentrations, without interfering with tyrosinase activity. These agents might act via acceleration of glutathione synthesis and inhibition of tyrosinase transfer.

Thiociot acid (α-lipoic acid), a disulfide derivative of octanoic acid, exerts several biological effects. It has been reported to prevent UV-induced oxidative damage, principally via the down-modulation of NF-κB activation. Additionally, it is known to inhibit tyrosinase activity, probably by chelating its copper ions.

**Inhibitors of inflammation-induced melanogenic response**

Pro-inflammatory mediators, such as interleukin-1α and endothelin 1 (ET-1), are generated by keratinocytes after exposure to inflammatory stimuli or UV exposure, and are capable of stimulating melanogenesis. Anti-inflammatory compounds might therefore prove useful for the prevention or treatment of post-inflammatory hyperpigmentation.

ET-1 exerts unique and powerful stimulatory effects on both DNA synthesis and melanization in human melanocytes. Topical application of *Matricaria chamomilla* extract inhibited UVB-induced pigmentation by avoiding ET-1-induced DNA synthesis but not interleukin-α-induced ET-1 production and tyrosinase activation.
Melanogenesis and hypopigmenting agents

Topical corticosteroids are representative anti-inflammatory agents. They have been included in several clinical trials for the treatment of melasma, and have also been used in attempts to reduce the irritation induced by hypopigmenting agents. They may act by the suppression of cytokines via the inhibition of NF-κB activation.

Glabridin, the principal component of the hydrophobic fraction of licorice extracts, reduced tyrosinase activity in B16 melanoma cells without affecting DNA synthesis, and inhibited UVB-induced skin pigmentation and erythema in guinea pigs. Its observed ability to inhibit cyclooxygenase activity and superoxide anion production suggests that its anti-inflammatory effect involves interference with the arachidonic acid cascade, and that protection against oxidative stress performs a key role in modulating melanogenesis.

Other signal regulators

The skin on the palms of the hands and the soles of the feet is less pigmented than the rest of the body skin. This is caused by the preferential expression of transcription factors by fibroblasts in the underlying dermis. Some investigators have found that the protein product of the gene dickkopf1 (DKK1) is a negative regulator of the Wnt signaling pathway, which decreases the levels of MITF, and therefore inhibits melanocyte growth and pigment production. Similarly, calpain inhibitors have been shown to cause marked reductions in both tyrosinase and its mRNA levels in B16 cells. Thus, treatment methods based on the specific stimulation of DKK1 or the inhibition of calpain represent potentially viable new approaches to the treatment of hypopigmentation.

Conclusions

Although the knowledge of melanocyte biology has made remarkable progress, the pathogenic mechanisms underlying acquired hyperpigmentation have yet to be completely elucidated. New mechanisms need to be explored to fully understand the biological basis of pigmentation, and to identify new and effective signal regulators. The dermal microenvironment has also been reported to influence epidermal pigmentation via dermal degeneration or vascular dilatation. These findings suggest that the environmental regulation of melanogenesis is a complex process. Different hypopigmenting agents have been discussed on the basis of a review of the literature, and on the authors’ clinical and research experiences.

Acknowledgments

This study was supported by a grant (A050432) from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Korea.

References


