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Review Article

Natural tyrosinase inhibitors for skin hyperpigmentation

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CONTENTS

Abstract

Skin hyperpigmentation can be a serious aesthetic problem with severe psychological impact. Skin color is determined by the type and quantity of melanin, a dark pigment produced by melanocytes. The initial rate-limiting steps of melanin biosynthesis are catalyzed by tyrosinase, a copper-containing binuclear enzyme. Because of its key role in melanogenesis, tyrosinase is an attractive target for the development of skin-whitening agents. Although a number of tyrosinase inhibitors have been reported in the literature, skin-whitening activities have been reported for surprisingly few compounds. This article presents a review of compounds that meet three criteria: 1) they possess confirmed skin-whitening activity; 2) they inhibit at least one aspect of tyrosinase function; and 3) they occur in nature.

Introduction

Skin hyperpigmentation can be a serious aesthetic problem. In Western countries, aesthetic-related concerns typically result from irregular pigmentation, including melasmas, lentigies, age spots, inflammatory hypermelanosis and trauma-induced hyperpigmentation. In Asia, the incentive for skin whitening is exacerbated by the traditional belief that whiter skin is the epitome of beauty and youth.

The color of human skin depends on the content and composition of the dark pigment melanin. Melanin is produced by melanocytes within specialized organelles, known as melanosomes, and then transferred to the neighboring keratinocytes. Although melanin synthesis involves several enzymes, the initial (and rate-limiting) steps of this process are catalyzed by the enzyme tyrosinase (E.C. 1.14.18.1) (1). The key role of tyrosinase in skin pigmentation is evidenced by mutations in the tyrosinase gene that lead to albinism (2). As such, tyrosinase remains an attractive target for designing various depigmenting agents.

Most of the known tyrosinase inhibitors directly interfere with the catalytic functions of this enzyme. Tyrosinase possesses an active site with two copper atoms, both of which are coordinated by three histidine residues (3). Mammalian tyrosinase catalyzes three steps of melanin biosynthesis: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), the oxidation of DOPA to dopaquinone and the oxidation of 5,6-dihydroxyindole to indolequinone (1). The tyrosine hydroxylase and DOPA oxidase activities may have different sensitivity to inhibitors (4). It is unclear whether these activities occur at the same catalytic site of the enzyme or involve separate sites (5). The inhibitors can act both in competitive and noncompetitive fashions. Competitive and noncompetitive inhibitors may interact with different sites of the enzyme, and in combination they can produce synergistic effects (6). Tyrosinase is an essential enzyme that occurs in all organisms, and tyrosinase extracted from mushrooms has been widely used to evaluate potential skin-whitening compounds. However, extrapolating mushroom tyrosinase data to the mammalian enzymes should be done with caution. Mammalian and mushroom tyrosinases have distinct catalytic properties (7) and sensitivities to inhibitors (8, 9).

In addition to direct enzyme inhibition, some depigmenting agents target tyrosinase by interfering with its expression and posttranslational processing. The mammalian tyrosinase promoter contains a highly conserved motif, termed the M-box. This element is regulated by a basic-helix-loop-helix-leucine-zipper protein, named microphthalamia-associated transcription factor (MITF) (10). Depigmenting agents that target MITF inhibit the transcription of tyrosinase (11-13). Certain depigmenting agents may also interfere with posttranslational maturation and compartmentalization of the enzyme. Human tyrosinase cDNA encodes for a 531-amino-acid type I

transmembrane protein containing five potential *N*-glycosylation sites and an *N*-terminal signal peptide that targets the nascent protein to the endoplasmic reticulum (14). From the endoplasmic reticulum, tyrosinase passes through the Golgi region, where it undergoes additional glycosylation and eventually reaches the target organelles, the melanosomes (5). Compounds that interfere with glysosylation result in aberrant retention of tyrosinase in the endoplasmic reticulum (15, 16). Some compounds can also inhibit melanin production by accelerating proteolytic degradation of tyrosinase (17, 18).

Tyrosinase inhibitors have been addressed from multiple aspects in several excellent reviews (19-22). The emphasis of this article is on naturally occurring compounds that directly or indirectly inhibit the function of mammalian tyrosinase and also possess confirmed skin-whitening activities.

The following section includes descriptions of natural tyrosinase inhibitors with confirmed skin-whitening effects. Molecular structures, mechanisms of action and potency of these compounds are summarized in Table I.

Competitive tyrosinase inhibitors

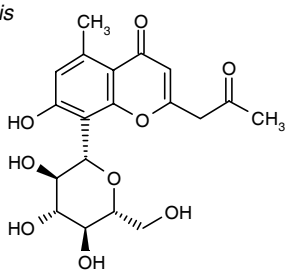
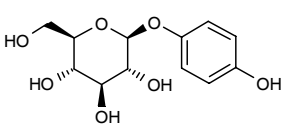
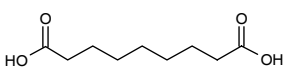
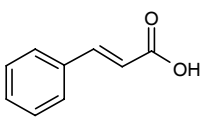
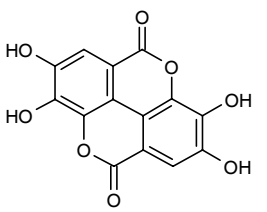
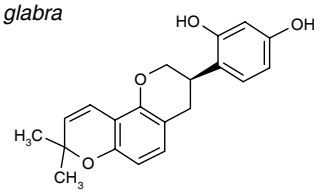
Arbutin (4-hydroxyphenyl-β-D-glucopyranoside), a glycosylated form of hydroquinone, is an active ingredient of the traditional Japanese medicinal plant Uvae Ursi. Although arbutin inhibits both tyrosine hydroxylase and DOPA oxidase activities of human tyrosinase, the inhibition of tyrosine hydroxylation activity is much more robust, with K_i values of 2.01 and 16.48 mM for tyrosine hydroxylase and DOPA oxidase activities, respectively. Both activities are inhibited in a competitive fashion (4). In experiments using intact human melanocytes, arbutin inhibited tyrosinase activity with an IC_{50} of 0.5 mM. Arbutin can be considered a prodrug that releases the hydroquinone moiety by a slow in vivo hydrolysis of the glycosidic bond (22). A randomized clinical trial demonstrated that arbutin inhibited UV-induced hyperpigmentation of human skin (23). Arbutin has very poor skin penetration (24), and the use of this agent is rather limited (25).

Hydroquinone (1,4-benzenediol) is produced naturally by many plants and insects. In a murine tyrosinase assay with L-DOPA as the tyrosinase substrate, hydroquinone inhibited the enzyme with an IC_{50} of 0.65 mM. In experiments using intact murine melanocytes, hydroquinone inhibited melanin formation with an IC_{50} of 10 μM (26). Significant cytotoxicity for hydroquinone was observed in these studies. Hydroquinone inhibits tyrosinase in a competitive fashion and may act as an alternative substrate that generates a colorless product (27, 28). In addition, hydroquinone inhibits melanogenesis by disintegrating epidermal melanocytes, interfering with melanosome formation and accelerating melanosomal degradation within keratinocytes (29). Although the depigmenting effects of hydroquinone were discovered more than 50 years ago (30), this compound is still considered a reference standard for new hypopigmenting agents. At a concentration of 4-5%, topical hydroquinone formulations are very efficient for melasma treatment, although they can have significant irritant effects. Lower concentrations of this compound are much less effective for melasma (31). Typically, hydroquinone is used in combinations with retinoids or steroids. The efficacy of hydroquinone has been confirmed in several clinical trials. For instance, one double-blind, placebo-controlled study involving 48 subjects demonstrated 40% total improvement of melasma after treatment with 4% hydroquinone, versus only 10% total improvement in the placebo group (32). Hydroquinone has considerable drawbacks, however. The compound is not very stable in formulation and has significant side effects, including irritant contact dermatitis, allergic contact dermatitis, postinflammatory hyperpigmentation, nail discoloration, permanent depigmentation and permanent discoloration (ochronosis) (31). In addition, hydroquinone is a suspected carcinogen (33). Because of these adverse effects, hydroquinone has been banned in Europe and Asia, and the ban of hydroquinone as an OTC skin-whitening agent is under consideration in the U.S. (25).

Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-4-pyranone) is a fungal metabolite produced by many species of *Aspergillus* and *Penicillium* (34). Kojic acid inhibited tyrosine hydroxylase activity of mammalian tyrosinases with IC_{50} values ranging from 42 to 400 μM (26, 35, 36). The potency of kojic acid in cell-based systems is rather low. The compound inhibited melanin production in human and murine melanocytes with IC_{50} values exceeding 200 μM (26, 36). Kojic acid acts as a competitive tyrosinase inhibitor (36). The mechanistic model of kojic acid action on tyrosinase has been drawn from the structure of catechol oxidase, an enzyme that has a similar structure of the active site as tyrosinase (37). According to the model, kojic acid acts as a copper chelator, forming a bridge between two cooper atoms in the tyrosinase active site. In a randomized, split-face trial for melasma, kojic acid was as effective as hydroquinone (38). In another randomized, split-face melasma trial, a formulation of glycolic acid and hydroquinone that contained 2% kojic acid was more effective than the formulation without kojic acid (39). The safety of kojic acid is a concern because this agent induces contact dermatitis (40) and can be mutagenic both in the Ames test and in CHO cells (41, 42).

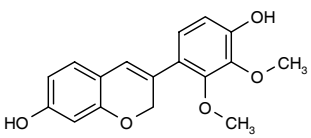
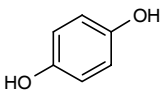
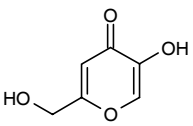
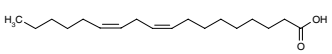
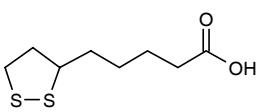
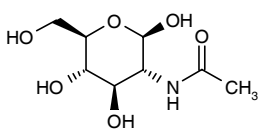
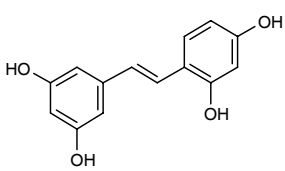
UP-302 (Nivitol™) (1-[2,4-dihydroxyphenyl]-3-[2,4-dimethoxy-3-methylphenyl]propane) is a dimethoxytolyl propylresorcinol compound isolated from the Asian medicinal plant *Dianella ensifolia* (43). Several compounds of the same class isolated from different plants also exhibited tyrosinase-inhibitory activity. UP-302 inhibited DOPA oxidase activities of mushroom and murine tyrosinases with a K_i value of 0.3 μM and an IC_{50} value of 12 μM, respectively. UP-302 is a competitive and reversible tyrosinase inhibitor. It inhibited melanin production in murine melanoma B16 cells and human primary melanocytes with IC_{50} values 15 and 8 μM, respectively. In a reconstructed skin model (MelanoDerm™) topical application of UP-302 at concentrations 0.05% and 0.1%

Table I: Natural tyrosinase inhibitors with confirmed skin-whitening effects.

Name	Representative source	Chemical structure	Potency	Mechanism(s) of action	Efficacy in skin depigmenting studies
Aloesin	*Aloe barbadensis* (*Aloe vera*)		K_i = 0.152 mM (human tyrosinase, L-DOPA as the substrate) (35) IC_{50} = 0.167 mM (melanin formation by B16-F1 cells) (35) IC_{50} = 0.7 mM (human tyrosinase, L-tyrosine as the substrate) (35)	Competitive tyrosinase inhibitor (35) Noncompetitive tyrosinase inhibitor (6, 44)	10% topical inhibited UV-induced human skin pigmentation (23)
Arbutin	*Arctostaphylos uva-ursi* (bearberry)		K_i = 16.48 mM (human tyrosinase, L-DOPA as the substrate) (4) K_i = 2.01 mM (human tyrosinase, L-tyrosine as the substrate) (4) IC_{50} = 0.5 mM (cellular tyrosinase activity, human melanocytes) (4)	Competitive tyrosinase inhibitor (4, 71)	10% topical inhibited UV-induced human skin pigmentation (23)
Azelaic acid	*Pityrosporum ovale* (yeast)		K_i = 2.73 mM (mushroom tyrosinase, L-tyrosine as the substrate) (29)	Direct mechanism: competitive tyrosinase inhibitor (59) Indirect mechanism: thioredoxin reductase inhibitor (60)	15-20% topical was effective in clinical trials for melasma (62-65), facial hyperpigmentation (67) and lentigo maligna (66)
Cinnamic acid	*Cinnamomum cassia* (cinnamon)		IC_{50} = 0.693 mM (murine tyrosinase, L-DOPA as the substrate) (68) IC_{29} = 0.5 mM (melanin formation by Melan-A cells) (68)	Tyrosinase inhibitor (68) Inhibits expression of tyrosinase (68)	1% topical inhibited UV-induced guinea pig skin hyperpigmentation (68)
Ellagic acid	*Punica granatum* (pomegranate)		IC_{50} = 182.2 μg/ml of the extract containing 90% ellagic acid (mushroom tyrosinase, L-tyrosine as the substrate) (46) $IC_{54.4}$ = 4 μM (melanin formation by B16 cells) (45)	Noncompetitive tyrosinase inhibitor; acts by chelating copper ions (45)	1% topical inhibited UV-induced guinea pig skin hyperpigmentation (45) 1 g/kg oral inhibited UV-induced guinea pig skin hyperpigmentation (46)
Glabridin	*Glycyrrhiza glabra* (licorice)		K_i = 0.38 mM (mushroom tyrosinase, L-tyrosine as the substrate) (47) K_i = 0.81 mM (mushroom tyrosinase, L-DOPA as the substrate) (47) IC_{55} = 10 μM (melanin formation by human G-361 melanocytes) (47)	Noncompetitive tyrosinase inhibitor (47) Inhibits expression of tyrosinase (48)	0.5% topical inhibited UV-induced guinea pig skin hyperpigmentation (48)

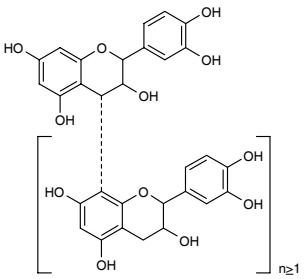
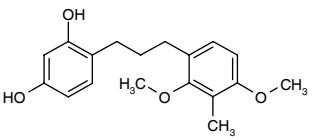
continuation

Table I (Cont.): Natural tyrosinase inhibitors with confirmed skin-whitening effects.

Name	Representative source	Chemical structure	Potency	Mechanism(s) of action	Efficacy in skin depigmenting studies
Haginin A	*Lespedeza cyrtobotrya* (bushy bushclover)		K_i = 1.48-2.17 mM (mushroom tyrosinase, L-tyrosine as the substrate) (12) IC_{50} = 3.3 and 2.7 mM (melanin formation by mouse Melan-A and human embryonic melanocytes, respectively) (12)	Noncompetitive tyrosinase inhibitor (12) Inhibits expression of tyrosinase and TRP1 (12)	1% topical inhibited UV-induced guinea pig skin hyperpigmentation (12)
Hydroquinone	*Juglans regia* (European walnut)		IC_{50} = 0.65 mM (murine tyrosinase, L-tyrosine as the substrate) (26) IC_{50} = 10 μM (melanin formation by mouse Melan-A melanocytes, cytotoxic) (26)	Competitive tyrosinase inhibitor and alternative tyrosinase substrate (27, 28)	4% topical was effective in clinical trials for melasma and postinflammatory hyperpigmentation (31, 32)
Kojic acid	*Aspergillus flavus* (fungal metabolites)		IC_{50} = 42–>100 μM (murine tyrosinase, L-tyrosine as the substrate) (26, 36, 37) IC_{50} = 400 μM (human tyrosinase, L-tyrosine as the substrate) (35) IC_{50} > 200 μM (melanin formation by human embryonic melanocytes and murine melanocytes Melan-A and B16) (26, 36)	Competitive tyrosinase inhibitor (36)	2% topical enhanced the effects of hydroquinone and glycolic acid in clinical trials for melasma (38, 39)
Linoleic acid	*Carthamus tinctorius* (safflower)		IC_{50} = 25 μM (melanin formation by B16 cells) (17)	Accelerates posttranslational degradation of tyrosinase protein (17)	0.5% topical reduced UV-induced guinea pig skin hyperpigmentation (18)
Lipoic acid	*Solanum tuberosum* (potatoes)		$IC_{60} \cong$ 200 μM (tyrosinase activity in the lysates obtained from the inhibitor-treated B16 cells, L-DOPA as the substrate) (13)	Inhibits expression of both tyrosinase and TRP1 (13)	1% topical reduced natural skin pigmentation in dark-skinned Yukatan swine and inhibited UV-induced hyperpigmentation in light-skinned Yukatan swine (13)
N-Acetylglucosamine, glucosamine	*Cancer rnagister* (Dungeness crab)		Prolonged treatment with 1 mg/ml of glucosamine resulted in almost 100% inhibition of both tyrosinase activity and melanogenesis in B16 cells (16)	Inhibits posttranslational processing of tyrosinase (16)	2% topical *N*-acetylglucosamine reduced skin hyperpigmentation in Japanese subjects and enhanced skin-whitening effect of niacinamide in Caucasian subjects (15)
Oxyresveratrol	*Morus alba* (common mulberry)		K_i = 0.43 μM (mushroom tyrosinase, L-tyrosine as the substrate) (36) K_i = 0.91 μM (mushroom tyrosinase, L-DOPA as the substrate) (51) IC_{50} = 52.7 μM (murine tyrosinase, L-tyrosine as the substrate) (36)	Noncompetitive and reversible tyrosinase inhibitor (36)	*Morus alba* extract containing oxyresveratrol inhibited UV-induced guinea pig skin hyperpigmentation (52)

continuation

Table I (Cont.): Natural tyrosinase inhibitors with confirmed skin-whitening effects.

Name	Representative source	Chemical structure	Potency	Mechanism(s) of action	Efficacy in skin depigmenting studies
Proantho-cyanidins	*Vitis vinifera* (grape seeds)		IC_{50} = 35 μg/ml (grape seed extract containing 89.3% of proantho-cyanidins; mushroom tyrosinase, L-DOPA as the substrate) (55)	Noncompetitive tyrosinase inhibitor (55)	1.1 g/kg oral administration of grape seed extract containing 89.3% of proantho-cyanidins inhibited UV-induced guinea pig skin hyperpigmentation (55)
UP-302 (Nivitol™)	*Dianella ensifolia* (Cerulean flaxlily)		K_i = 0.3 μM (mushroom tyrosinase, L-DOPA as the substrate) (43) IC_{50} = 12 μM (murine tyrosinase, L-DOPA as the substrate) (43) IC_{50} = 15 and 8 μM (melanin formation by B16 cells and human embryonic melanocytes, respectively) (43)	Competitive and reversible tyrosinase inhibitor (43)	0.05% topical reduced spontaneous pigmentation of reconstructed human skin (MelanoDerm™) more efficiently than 1% kojic acid (43)

exhibited stronger depigmenting activity than 1% kojic acid. As such, UP-302 appears to be one of the most potent skin-whitening agents reported to date.

Noncompetitive tyrosinase inhibitors

Aloesin (2-acetonyl-8-β-D-glucopyranosyl-7-hydroxy-methylchromone) is a *C*-glycosylated chromone isolated from *Aloe* plants. Aloesin inhibited tyrosine hydroxylase activity of human tyrosinase with an IC_{50} of 0.7 mM and DOPA oxidase activity with a K_i of 0.152 mM (35). In experiments using intact murine melanocytes aloesin inhibited melanin formation with an IC_{50} of 0.167 mM. According to one report, aloesin inhibited tyrosinase in a competitive fashion (35), whereas other authors reported a noncompetitive mechanism of inhibition (6, 44). In agreement with a noncompetitive mode of action, aloesin demonstrated synergistic activity with the competitive tyrosinase inhibitor arbutin (6). In a randomized clinical trial, aloesin inhibited UV-induced hyperpigmentation in dose-dependent fashion (23). Aloesin permeates the skin slowly and is used as a depigmenting agent primarily in combinations with arbutin or deoxyarbutin (25).

Ellagic acid (4,4´,5,5´,6,6´-hexahydroxydiphenic acid 2,6,2´,6´-dilactone) is a naturally occurring polyphenol found in a variety of plants, including pomegranate, strawberry and green tea. At a relatively low concentration of 4 μM, ellagic acid reversibly inhibited both tyrosine hydroxylase activity and melanin production in murine melanoma B16 cells. Ellagic acid inhibited mushroom tyrosinase noncompetitively, apparently by chelating copper ions in the enzyme's active center. The inhibition was partially reversed by the inclusion of copper salts into the reaction buffer. Topical application of 1% ellagic acid inhibited the development of UV-induced hyperpigmentation of guinea pig skin, and also accelerated whitening of the pre-existing pigmentation. In these experiments the effect of 1% ellagic acid was comparable to 1% hydroquinone (45). Ellagic acid was active not only on topical application but also upon oral administration. Feeding guinea pigs with pomegranate extract enriched with 90% ellagic acid (1 g/kg) prevented UV-induced skin pigmentation. This treatment also significantly reduced the number of DOPA-positive melanocytes (46). It would be interesting to see whether similar effects can be observed in human clinical trials.

Glabridin (4-[3,4-dihydro-8,8-dimethyl-2*H*,8*H*-benzo-[1,2-*b*:3,4-*b*´]dipyran-3-yl]-1,3-benzenediol) is found in the extracts of licorice roots. Glabridin inhibited the tyrosine hydroxylase activity of mushroom tyrosinase more potently than the DOPA oxidase activity, with K_i values 0.38 and 0.84 mM, respectively. Both activities were inhibited in a noncompetitive fashion. Since glabridin is also capable of inhibiting the expression of tyrosinase, this agent is more potent in cell-based systems than in cell-free enzyme activity assays. Experiments using human malignant melanoma G-361 cells demonstrated that concentrations as low as 10 μM glabridin were sufficient to suppress melanin production more than twofold (47). Comparable results were obtained in B16 murine melanocytes. As glabridin is capable of suppressing the entire DNA synthesis, the selectivity of its effect on tyrosinase expression

is not clear at this point. In guinea pig experiments, 0.5% glabridin inhibited UV-induced skin hyperpigmentation (48). The drawbacks of glabridin include poor skin penetration and low stability in formulation.

Oxyresveratrol (2',3,4',5-tetrahydroxystilbene) is a hydroxystilbene compound isolated from Ramulus mori, the young twigs of the white mulberry tree. Although several other stilbene compounds also possess tyrosinase-inhibitory activities (36, 49, 50), to the best of our knowledge only oxyresveratrol has been confirmed as a skin-whitening agent. Oxyresveratrol inhibited both the tyrosine hydroxylase and DOPA oxidase activities of mushroom tyrosinase with K_i values of 0.43 and 0.91 μM, respectively (36, 51). Tyrosine hydroxylase activity of murine tyrosinase was inhibited with an IC_{50} of 52.7 μM. Oxyresveratrol inhibited tyrosinase in a noncompetitive and reversible fashion (36). Oxyresveratrol-containing Ramulus mori extracts inhibited UV-induced guinea pig skin hyperpigmentation (52). Since the exact composition of plant extracts used in this study has not been defined, the in vivo potency of oxyresveratrol is not clear at this point.

Proanthocyanidins are oligomers of tannins that consist of multiple polyhydroxyflavan-3-ols, (+)-catechin and/or (–)-epicatechin. These compounds are present in significant amounts in grape seeds. In the acidic environment of the gastric milieu these polymers rapidly decompose to lower oligomers and (–)-epicatechin (53). Orally administered proanthocyanidins tend to accumulate in certain tissues, including skin (54). Grape seed extracts containing 89.3% proanthocyanidins inhibited the DOPA oxidase activity of mushroom tyrosinase noncompetitively with an IC_{50} of 35 μg/ml. Similarly, 25 and 50 μg/ml of grape seed extract containing 54.4% of proanthocyanidins decreased the melanin content in B16 cells to 65.6% and 59.9%, respectively. After 8 weeks of feeding guinea pigs with grape seed extract containing 89.3% proanthocyanidins (at 1.1 g/kg/day), both the extent of UV-induced skin hyperpigmentation and the number of DOPA-positive melanocytes were significantly reduced (55).

Compounds that decrease the levels of tyrosinase protein

Lepidium apetalum, an extract from a Chinese medicinal plant, appears to inhibit tyrosinase function at the transcriptional level (11). By itself, the extract did not affect tyrosinase expression in monocultures of human melanoma HM3KO cells. However, incubation of melanoma cells with conditioned media obtained from the keratinocytes pretreated with the *Lepidium* extract significantly inhibited melanin production. The inhibition was accompanied by a decrease in production of both tyrosinase and MITF. These inhibitory effects were observed at both the protein and mRNA levels. These observations suggested that, in response to the *Lepidium* extract, keratinocytes produced certain factors that inhibit the function of melanocytes. In particular, the authors found that keratinocytes treated with *Lepidium* increased the production of IL-6, a cytokine known to inhibit melanogenesis (56). The authors directly confirmed the involvement of IL-6 by using a neutralizing antibody to this cytokine. A 2% *Lepidium* extract suppressed UV-induced hyperpigmentation of guinea pig skin. In these experiments the extract was as efficient as 2% hydroquinone. The identification of active ingredients in the *L. apetalum* extract should help in understanding the mechanism of its action.

Linoleic acid (*cis*-9,12-octadecadienoic acid) is an unsaturated omega-6 fatty acid. It is a major component of vegetable oil extracted from various plants, including safflower, poppy seed and walnuts. Metabolic labeling of murine melanoma B16 cells demonstrated that 25 μM of linoleic acid accelerated the degradation of tyrosinase, whereas the levels of tyrosine-related proteins TRP1 and TRP2 were not affected. Interestingly enough, palmatic acid, which is a saturated fatty acid, exhibited just the opposite effect by increasing the cellular level of tyrosinase and melanin production (17). Topical application of 0.5% linoleic acid significantly accelerated the lightening of pre-existing UV-induced hyperpigmentation of guinea pig skin. α-Linolenic acid (omega-3 fatty acid) and oleic acid (monounsaturated omega-9 fatty acid) exhibited similar, albeight weaker, depigmenting effects (18).

Lipoic acid (5-[(3*R*)-dithiolan-3-yl]pentanoic acid) is a sulfur-containing carboxylic acid produced by mitochondria. This compound is prevalent in the leaves of plants containing mitochondria, nonphotosynthetic plant tissues, as well as animal tissues. Dihydrolipoic acid (6,8-dimercaptooctanoic acid) is a reduced form of lipoic acid. Pretreatment of murine B16 melanocytes with lipoic acid reduced the DOPA oxidase activity of cell homogenates with an IC_{60} of approximately 200 μM. In B16 and Melan-A cells both lipoic and dihydrolipoic acids modulated MITF promoter activity and reduced levels of MITF, tyrosinase and TRP1 (13). The inhibitory effects of these compounds on the MITF promoter activity were confirmed using an MITF-luciferase reporter construct and MITF northern blotting. The in vivo effects of both lipoic and dihydrolipoic acids were investigated in Yucatan swine. Topical application of these compounds (at concentrations of 1%) reduced the coloration of dark-skinned animals and prevented UV-induced tanning of light-skinned animals. Both compounds exhibited comparable activity. The molecular mechanism of the modulation of MITF promoter activity is not clear at this point.

N-Acetylglucosamine (2-[acetylamino]-2-deoxy-D-glucose) is the monomeric unit of the polymer chitin, which forms the outer coverings of insects and crustaceans. Glucosamine is a deacetylated form of *N*-acetylglucosamine. Both amino sugars occur in all human tissues as they are the key structural components of glycosylated proteins, glucosaminoglycans and glycolipids. Prolonged treatment of B16 melanocytes with 1 mg/ml of glucosamine resulted in almost complete loss of both tyrosinase activity and melanin production (16). Glucosamine and *N*-acetylglucosamine inhibit the function of tyrosinase by altering posttranslational processing of tyrosinase protein. *N*-Glycosylation plays an important role in both the stabilization of this protein in the endo-

plasmic reticulum and its subsequent transportation to melanosomes. Both *N*-acetylglucosamine and glucosamine were well tolerated in skin applications. As *N*-acetylglucosamine is more stable than glucosamine and readily penetrates human skin, the former compound was tested in two clinical trials. An 8-week, double-blind, split-face clinical study among female Japanese subjects demonstrated that topical application of 2% *N*-acetylglucosamine resulted in statistically significant improvement of facial hyperpigmentation. In another, 8-week, double-blind, split-face clinical study among female Caucasian subjects, 2% *N*-acetylglucosamine was tested in combination with 4% niacinamide, an inhibitor of melanosome transfer. The results of this trial showed that niacinamide combined with *N*-acetylglucosamine was more potent than niacinamide alone (15).

Oolong tea is a traditional Chinese tea that contains certain unique polyphenols. These compounds, known as oolong tea polymerized polyphenols (OTPPs), are generated in the process of fermentation and are not contained in either green or black tea. The oolong tea extract inhibited melanin production by murine B16 melanocytes with an IC_{50} of approximately 50 mg/ml (57). The inhibition of melanogenic activity was accompanied by a decrease in the cellular tyrosinase protein and mRNA levels. In the same study, feeding UV-irradiated guinea pigs with oolong tea extract (138 mg/animal/day of polyphenols) reduced the number of DOPA-positive melanocytes approximately twofold in comparison with the control group. The exact chemical composition of the extract used in these studies has not been defined.

Compounds with mixed mode of action

Azelaic acid (1,7-heptanedicarboxylic acid) is a 9-carbon dicarboxylic acid isolated from the yeast strain *Pityrosporum ovale (58)*. Azelaic acid appears to inhibit skin pigmentation by a dual mechanism. This compound acts as a direct competitive inhibitor of mushroom tyrosinase with modest potency (K_i = 2.73 mM). In addition, azelaic acid inhibits another enzyme, thioredoxin reductase, with high potency (K_i = 12.5 μM) (59). A model was proposed where inhibition of thioredoxin reductase leads to intracellular accumulation of reduced thioredoxin, which is a potent inhibitor of tyrosinase (60). Furthermore, inhibition of thioredoxin reductase suppresses the production of deoxyribonucleotides, which may explain the ability of azelaic acid to inhibit total DNA synthesis in melanomas (61). The in vivo efficacy of azelaic acid was confirmed in a number of clinical trials. In one single-blind, split-face study, 20% azelaic acid alone was effective in the treatment of melasma (62). In another 24-week, double-blind melasma study in 329 women, 20% azelaic acid cream yielded good or excellent results in 65% (63). In two melasma trials, the depigmenting effects of azelaic acid were directly compared with hydroquinone. In one randomized, double-blind study, 20% azelaic acid was superior to 2% hydroquinone (64). Similarly, in another melasma study, 20% azelaic acid was superior to 2% hydroquinone and as effective as 4% hydroquinone (65). Azelaic acid was also effective against lentigo maligna. In a small-scale (3 subjects) open-label study, 15% azelaic acid produced remarkable clinical and histological effects that were maintained for up to 2 years after cessation of treatment (66). Finally, in a randomized, double-masked, parallel-group study of facial hyperpigmentation, 20% azelaic acid produced significantly greater decreases in pigmentary intensity than the vehicle (67).

Cinnamic acid (*trans*-3-phenylacrylic acid) is one of the major components of *Cinnamomum cassia*, an evergreen tree native to southern Asia. It is used primarily for its aromatic bark, known as “cinnamon”. Cinnamic acid inhibited the DOPA oxidase activity of murine tyrosinase with an IC_{50} of 0.693 mM (68). Treatment of Melan-A cells with 500 μM cinnamic acid reduced melanin production by 29%. The inhibition of melanin production was accompanied by a decrease in the levels of tyrosinase protein, suggesting a dual mechanism of action. In the same study, the effects of cinnamic acids were tested on UV-induced hyperpigmentation of guinea pig skin. In these experiments, topical treatment with 1% cinnamic acid significantly accelerated skin whitening.

Haginin A (4´,7-dihydroxy-2´,3´-dimethoxyisoflavone) was isolated from the branch of the Asian shrub *Lespedeza cyrtobotrya* (12). Haginin A inhibited tyrosine hydroxylase activity of mushroom tyrosinase noncompetitively (K_i = 1.48-2.17 mM), and it inhibited melanin production with IC_{50} values of 3.3 and 2.7 μM, respectively, in murine Melan-A cells and human embryonic melanocytes. Cytotoxicity was observed at a concentration approximately 5 times higher than the concentration at which the depigmenting effect occurred. Haginin A inhibits pigmentation by a dual mechanism. In addition to directly inhibiting tyrosinase, this agent reduced protein levels of two key enzymes involved in melanin production: tyrosinase and TRP1. Furthermore, the level of MITF, the transcription factor that regulates the expression of both tyrosinase and TRP1 (69), was also reduced. The level of MITF mRNA was not affected by haginin A, suggesting that this agent downregulates MITF posttranscriptionally. The effects of haginin A were also tested on UV-induced hyperpigmentation of guinea pig skin. In these experiments, haginin A accelerated skin whitening more than 3 times. In addition, haginin A exhibited remarkable depigmenting effects on zebrafish embryos.

Perspectives

The development of future tyrosinase inhibitors includes both modifying existing agents and searching for new modalities.

To alleviate some adverse side effects of hydroquinone, the monomethyl ether of this compound has been synthesized. This derivative, known as mequinol, is less cytotoxic and irritating than hydroquinone (70).

To increase the efficacy of arbutin, two synthetic derivatives have been developed. The optical isomer of

arbutin, 4-hydroxyphenyl-β-D-glucopyranoside (α-arbutin), is 20 times more potent toward human tyrosinase than β-arbutin (71). Another synthetic derivative of arbutin, deoxyarbutin, a compound that lacks all five hydroxyl groups in its sugar backbone, exhibited a 350-fold lower K_i than arbutin in a mushroom tyrosinase assay. Deoxyarbutin was also much more potent than arbutin both in a guinea pig skin model and in human clinical trials (24).

In an attempt to increase the potency of kojic acid, a synthetic derivative was generated where two pyrone rings of kojic acid were linked together by an ethylene spacer. The dimer was 8 times more potent than monomeric kojic acid in the tyrosinase inhibition assay. The compound was also more effective than kojic acid in inhibiting melanin synthesis in intact cells (72). Another approach to increasing the potency of kojic acid involved modifications that increase its skin permeation. The derivative, kojyl-APPA, was about 8 times more efficient in skin penetration than kojic acid (73).

One of the emerging approaches to inhibiting the function of tyrosinase involves agents that suppress transcription of the tyrosinase gene. Often these compounds decrease the levels of MITF, a transcriptional factor that regulates expression of both tyrosinase and several other melanogenesis-related genes (69). Some compounds in this class inhibit the transcription of the *MITF* gene (13, 74, 75). Of these, a traxastane-type triterpene isolated from the flowers of *Arnica montana* inhibited melanin production in B16 cells with nanomolar potency (74). Inhibitors of MITF transcription seem to interfere with upstream steps of signal transduction pathways that activate the MITF promoter and therefore may not be selective. Compounds that decrease levels of MITF posttranslationally, such as haginin A (12), are more likely to be selective. MITF binds a conservative motif, the M-box, that is shared by promoter sequences of several melanogenic proteins. It is conceivable, therefore, that future depigmenting agents capable of directly inhibiting binding of MITF to the M-box would act even more selectively.

Commonly used skin-whitening formulas often combine several depigmenting agents with different modes of action (31). As such, compounds capable of targeting multiple stages of melanogenesis are likely to have additional advantages. For instance, one recently reported compound, 4,4'-dihydroxybiphenyl, targets at least three processes involved in melanogenesis: activity of the tyrosinase enzyme (76), expression of the tyrosinase protein and oxidation of glutathione (75).

Conclusions

The potential withdrawal of hydroquinone from U.S. OTC markets and its removal from European and Japanese markets has prompted interest in novel skin-whitening agents. Although a number of tyrosinase inhibitors have been reported in the literature, surprisingly few compounds have been confirmed to possess skin-whitening activities in clinical settings. Among the factors that preclude the use of seemingly potent tyrosinase inhibitors in the clinic are skin irritation, skin toxicity, limited solubility, low stability in formulation, poor skin penetration and undesired colors of the active compounds. With the first crystal structure of tyrosinase recently determined (3) and with the increase in our understanding of the mechanisms that regulate the expression and processing of tyrosinase in mammalian cells, new tyrosinase inhibitors with improved safety and efficacy profiles are likely to emerge in the foreseeable future.

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