

Skin-lightening products revisited

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Synopsis

Skin colour typology depends on the amount and location of its chromophores. Among them, eumelanins derived from 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and 5,6-dihydroxyindole (DHI), and pheomelanins are of utmost importance. These biomolecules result from the multi-step enzymatic and non-enzymatic conversion of tyrosine into melanins. Pigmentation disorders are multiple and depend on alterations in the density in active melanocytes, and on specific abnormalities of any of the complex melanogenesis mechanisms. This review presents some of the main skin-lightening agents with respect to their mechanisms of action and side-effects. Some of the novel compounds may lead to new perspectives in the fields of dermatology and cosmetology. The methods commonly used to assess efficacy of skin-lightening products rely on *in vitro* models including cell-free enzymatic assays, melanocyte cultures and reconstructed epidermis bioassays. Animal models have little relevance. By contrast, human testing with the support of instrumental evaluations is the most informative.

Résumé

La couleur de la peau et la typologie dépendent de la quantité et de la localisation de ses chromophores. Parmi ceux-ci, les eumélanines dérivées des 5,6-dihydroxyindole-2-carboxylique acide (DHICA) et 5,6-dihydroxyindole (DHI) et les phaeomélanines sont de la plus grande importance. Ces biomolécules résultent

de la conversion enzymatique et non enzymatiques en plusieurs étapes de la tyrosine en mélanines. Les troubles de la pigmentation sont multiples et dépendent d'altérations dans la densité de mélanocytes actifs et d'anomalies spécifiques touchant l'un ou l'autre étape du processus complexe de la mélanogénèse. Cette revue présente quelques agents dépigmentants en considérant leurs mécanismes d'action et leurs effets secondaires. Certains des nouveaux composés ouvrent de nouvelles perspectives dans les domaines de la dermatologie et de la cosmétologie. Les méthodes visant à évaluer l'efficacité de produits dépigmentants font appel à des modèles *in vitro* incluant des bioessais enzymatiques, des cultures de mélanocytes et l'épiderme reconstruit. Les modèles animaux sont peu pertinents. En revanche, les tests sur volontaires humains s'appuyant sur des évaluations instrumentales sont les plus informatives.

Introduction

Skin colour results from the presence and ratio of several chromophores in the skin [1, 2]. Oxyhaemoglobin (bright red), reduced haemoglobin (bluish red) and bilirubin (yellow) are found in the small blood vessels of the dermis. Eumelanin (deep brown), pheomelanin (yellow to reddish brown) and sometimes carotenoids (yellow) are the main pigments of the epidermis. Pigmentary disorders are numerous and occur as a result of both genetic and environmental factors. Among these disorders, hypermelanosis is quite frequent and particularly troublesome in dark-skinned individuals. Current topical hypopigmenting agents present on the market target melanocytes, the diverse steps in melanogenesis and the melanin transfer to keratinocytes. Regardless of the personal motivation by individuals to alter their appearance, the psychological and socio-economic impacts of

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skin pigmentation and depigmentation are great in humans.

Melanization process

Melanins are pigmented biopolymers that impart skin typology and tan. They are synthesized by the dendritic melanocytes dispersed at the dermo-epidermal junction. Several genes are involved in regulating melanocyte biology and melanogenesis [3, 4]. Melanin synthesis takes place in membrane-bound organelles termed melanosomes, which contain specific enzymes controlling the production of the pigments.

The first and rate-limiting step of melanin formation is mediated by tyrosinase. This enzyme catalyses the hydroxylation of tyrosine into 3,4-dihydroxyphenylalanine (DOPA) and the subsequent oxidation of DOPA into DOPAquinone. By auto-oxidation and spontaneous cyclization, DOPA produces 5,6-dihydroxyindole (DHI) melanin (Fig. 1). Other melanogenic enzymes are involved in the multi-step pathway. In the presence of tyrosinase-related protein 2 (TRP-2), also known as DOPAchrome tautomerase, DOPAchrome is tautomerized to 5,6-dihydroxyindole-2-carboxylic acid (DHICA). TRP-1, also known as DHICA oxidase, promotes further oxidation and polymerization of DHICA melanins. Moreover, if a sulphhydryl donor (glutathione or cysteine) is available when DOPAquinone is generated, cysteinylDOPA can be formed. Pheomelanin is then produced by further oxidation, cyclization and polymerization.

Melanization of the epidermis is therefore a dynamic event involving many regulating factors. DHICA-derived melanins are brown in colour and DHI-derived melanins are black. Both are eumelanins and provide a better photoprotection than the yellowish-red pheomelanins.

According to the type of melanin synthesized, ellipsoidal eumelanosomes or spherical pheomelanosomes are formed. Then, the mature organelles migrate towards the extremities of the melanocyte dendrites where they are transferred to the surrounding keratinocytes. The protease-activated receptor 2 (PAR-2), which is expressed on keratinocytes but not on melanocytes, is involved in this transfer [5].

The resulting pigmentation is finally cleared by degradation of melanin during the ascent of the keratinocytes towards the outer stratum corneum. The remaining melanin pigments are shed with desquamation.

If the melanocyte density and distribution are altered or if one step of the melanogenesis pathway fails, disorders of pigmentation may appear. One example is given by the typical pigmentary changes appearing during intrinsic ageing and photoageing [6–9]. A decrease in the melanocyte number of about 10% per decade is observed in sun-protected areas after the 3rd decade of age. By contrast, chronic exposure to sunlight may increase the number and the activity of some clusters of melanocytes. In these circumstances, it is conceivable that some melanocytes are stimulated while others are suppressed. The unevenness of these modifications results in a spotty pattern of pigmentation, with areas of hyperpigmentation alternating with those of hypopigmentation [6, 8, 9].

The darker the innate skin colour, the more the presence of pigmentation disorders. The affected individuals often use depigmenting agents to unify their skin colour. Moreover, some dark-skinned women who want to obtain a lighter complexion use or abuse skin-lightening products. Searching for more effective and safe depigmenting agents in the wake of cultural and medical demand has increased over recent years. Nevertheless, the current status is far from being satisfactory.

Given the complexity of the melanization process, the active products might inhibit the melanin synthesis itself [10–12], the melanosome transfer [6, 8, 13, 14] or activate desquamation [15] in order to remove the superficial load in melanins. The induction of the melanogenesis process may also be prevented by neutralization of the endogenous and exogenous stimuli, such as ultraviolet radiations (UVR) [16–18], free radicals, reactive oxygen species [19], inflammatory mediators, some cytokines [20] and growth factors [21].

Biologic regulation of the melanization process

Cyclic adenosine monophosphate (cAMP) plays a key role in melanogenesis [22], and several pathways lead to its synthesis.

Keratinocytes produce pro-opiomelanocortin (POMC)-derived peptides including the α -melanocyte-stimulating hormone (α -MSH) and the adrenocorticotrophic hormone (ACTH) [23, 24]. Both of them can activate melanogenesis after their binding to their receptor MC1R [18, 25, 26] located at the melanocyte surface. This G protein-coupled receptor activates the adenylate cyclase to produce cAMP.

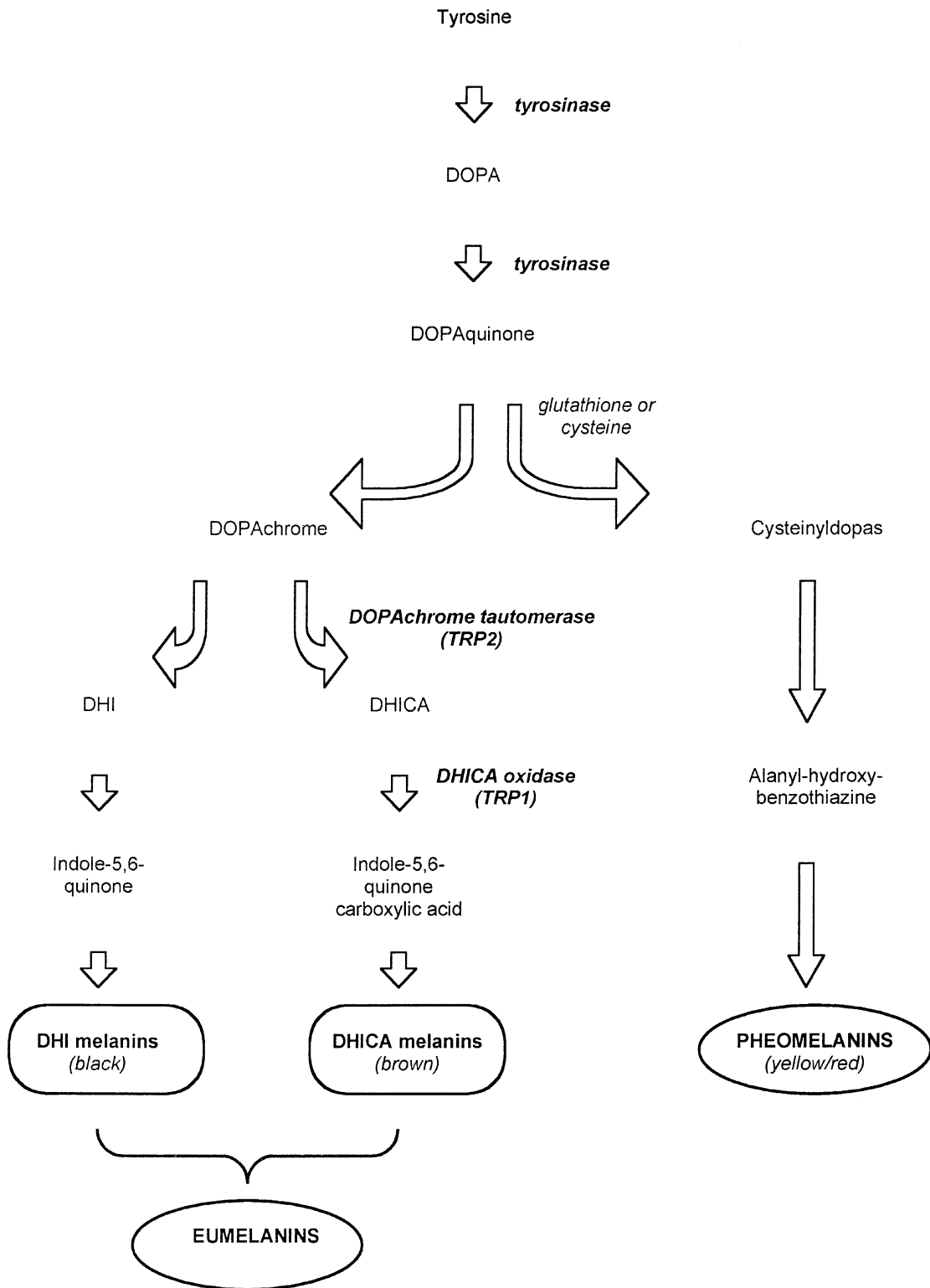


Figure 1 Melanogenic pathway. Simplified overview.

The agouti signal protein (ASP), a paracrine-signalling molecule produced from the agouti locus, has an antagonistic role to α -MSH. ASP would block the binding of α -MSH to the MC1R [27]. It thus decreases melanin synthesis and contributes to the production of pheomelanin rather than eumelanin [28].

In case of inflammation, prostaglandin E2 (PGE2) produced by keratinocytes stimulates tyrosinase activity and melanin synthesis. This inflammatory mediator also binds to the G protein-coupled receptors and therefore acts via the cAMP pathway [22]. Among inflammatory mediators, histamine may activate protein kinase A via H_2 receptors and therefore stimulates melanin synthesis in human normal melanocytes [29].

Nitric oxide (NO) plays a part in UV-induced melanogenesis [30]. UVR activate NO synthase and the resulting NO produced by melanocytes and keratinocytes activates the melanocytic-soluble adenylate cyclase. This leads to higher concentrations in cyclic guanosine monophosphate (cGMP), which in turn stimulate melanogenesis via the enhancement of tyrosinase expression and activity.

Several growth factors act on melanocyte proliferation and/or activity [22]. Among them, endothelin-1 (ET-1), which is produced by keratinocytes, stimulates both tyrosinase expression and growth of the neighbouring melanocytes. The endothelin-receptors are located at the melanocyte surface. These G protein-coupled receptors activate the protein kinase C pathway, which increases the intracellular calcium (Ca^{2+}) level.

Fatty acids may also regulate melanogenesis. Linoleic acid decreases melanin synthesis, possibly by proteolytic degradation of tyrosinase [31, 32]. By contrast, palmitic acid increases melanogenesis.

In sum, many factors can regulate melanogenesis, including various cytokines (IL-1, IL-6, TNF- α , . . .), growth factors (ET-1, bFGF, . . .) and inflammatory mediators (leukotrienes, prostaglandins) [20]. UVR are notably responsible for the production of many of them, and therefore act on the many facets of melanin synthesis, melanosome transfer, melanocyte proliferation, differentiation, and dendricity [17].

Screening of skin-lightening compounds

Pharmacological inhibitors of tyrosinase or other melanogenesis pathway targets may serve as topical inhibitors of melanogenesis resulting in skin

hypopigmentation. Several methods are available to assess the effects of potential skin-lightening products [11, 33, 34].

Enzymatic and cell culture methods

The *in vitro* mushroom tyrosinase inhibition assay is one basic step to assess the direct effect of a given skin lightener on tyrosinase activity [35]. This assay is more relevant when performed using purified mammalian tyrosinase or recombinant enzymes [11, 34]. As mentioned above, the substrate of the enzyme is L-tyrosine and the reaction requires the presence of the cosubstrate, L-DOPA. The activity of tyrosinase is quantified following the detection of DOPACHrome at 475 nm [33]. Other cell-free enzymatic assays can be performed, for instance to test, TRP-2 and TRP-1 activities.

However, any skin-lightening compound that inhibits tyrosinase or other enzymes must penetrate the melanocyte in order to perform its activity. The cellular surroundings may also influence the effect of the product. This might lead to false-positive responses. Moreover, there is a risk of false-negative response if the product has an indirect action on tyrosinase or exerts its activity on any other step of the melanin formation. Many inhibitors identified in cell-free enzymatic assays are likely to have some toxicity or delivery problems in cell-based assays.

A further screening step of new compounds can be performed on melanocytes maintained in culture. Currently, the most convenient models seem to be immortalized mouse- and melanoma-derived melanocytes. The relevant parameters are the effects on growth and viability of cells, melanin content and melanin synthesis [34]. Normal human melanocytes may also serve as a good model, provided the experimental conditions are well-controlled [33]. The compound is added to the culture either before melanogenesis stimulation (to assess its abilities to inhibit melanin synthesis) or after cells have produced a large amount of melanin (to assess its abilities to accelerate pigment degradation or elimination).

As melanogenesis often occurs under the mediation of the neighbouring keratinocytes, it is preferable to use cocultures of melanocytes and keratinocytes. For example, melanin synthesis may be quantified by bioassays using diethylaminoethyl-cellulose membrane filters [36] after introduction of the skin-lightening compound in cocultures of melanocytes and keratinocytes. The effects of new

compounds on photo-induced melanogenesis may also be assessed using these cocultures and UVR.

Reconstructed human epidermis

A more accurate model for assessing efficacy of pigment modifiers is the reconstructed epidermis, prepared from human keratinocytes and melanocytes [37]. Melanocytes can be collected from donors of different ethnic origins [38]. Coseeding these melanocytes and keratinocytes on a de-epidermized dermis gives rise to a multi-layered stratified and pigmented epidermis reflecting the skin phenotype of the donor. Another experimental model is offered by human skin maintained in survival. Exposure of the models to a UV solar simulator induces melanin synthesis and subsequent tanning, which allows assessment of the effects of lightening products or UV filters on the photo-induced pigmentation. Changes in skin colour can be quantified by instrumental assessments as described hereafter.

Animal models

Currently, available animal models for hypopigmenting efficacy testing are inadequate [11]. The characteristics of rodent skin and hair growth are different from human skin, particularly when epidermal melanogenesis is concerned.

Efficacy testing

Efficacy of skin-lightening products should be rated according to the uses and needs of the populations, which vary in different markets according to races and cultures. In general, these products are desired by Caucasian and Asian people to treat hyperpigmented spots. People with darker skin use them in order to produce whiter or Caucasian-like skin tone, which is considered to be more desirable in some societies. Hence, the intended uses range from genuine therapy to beauty-enhancing products.

In vivo human trials

Studies are performed on human volunteers under UV irradiation in order to determine the efficacy of formulations to inhibit the induction of photo-induced pigmentation and to measure the depigmentation rate [33]. The evaluation is performed using instrumental quantifications.

Clinical evaluations

In case of pigmented lesions, clinical trials should be performed over a few months [33]. In general, the products are applied twice daily for 2 months. A control area similar to the treated area must be secured. The studied parameters may include the number of pigmented spots, their surface and the intensity of their colour. Rating scales and clinical scores may be used, but these assessments by the evaluators remain subjective. The volunteers themselves may also assess the effects of the products. These evaluations are not always reliable unless a great number of subjects participate in the trial.

Instrumental evaluations

Objective instrumental evaluation of the efficacy of lightening products is recommended, particularly because these products exert their effects slowly. Bioengineering techniques are useful and new instrumental methods have been developed in recent years.

Colorimetric measurements

The skin colour intensity may be quantified by reflectance tristimulus colorimetry. The 'Commission Internationale de l'Eclairage' (CIE) defined in 1976 the CIELAB colour space system, widely used in dermatology and cosmetology to assess the skin colour [2, 39–41]. The three dimensions of this space represent three colorimetric parameters. One parameter defines the luminancy L^* on a scale ranging from 0 (black) to 100 (white). The L^* parameter is helpful to evaluate skin lightening or darkening. The more the skin is light, the more the L^* value is high. The two other parameters, namely chromaticities a^* and b^* , combine hue and chroma, and describe the colour wheel by two orthogonal axes that are perpendicular to the L^* axis. Chromaticity a^* is the red (+100) to green (-100) axis, and b^* the yellow (+100) to blue (-100) axis.

Narrow-band reflectance spectrophotometry explores the specific absorption wavelengths by the two main skin chromophores, melanin and haemoglobin [1, 2, 42–46]. The melanin index M is directly correlated to skin melanization, while the erythema index E quantifies the haemoglobin amount.

From these two colour assessment methods, the luminancy L^* and the M index are helpful in the assessment of the efficacy of skin-lightening products. However, the best description is probably given

by combining the L^* and b^* parameters [47] in the so-called individual typology angle (ITA°) following:

$$\text{ITA}^\circ = \text{ArcTangent}[(L^* - 50/b^*)] \times 180 \times \Pi^{-1}$$

Visualization methods

Photography is convenient in many clinical trials because image-recording methods allow to objectivate the activity of a product on pigmented spots. However, this method is largely dependent on many technical and methodological drawbacks. Reproducibility of acquired images requires skilled experimentalists and controlled procedures in special facilities.

The integration sphere consists of an annular visible light allowing the evaluation of the colour homogeneity, for example, of the face.

Controlled UV photographs are often more informative. The emitted UV radiation (365 nm) is attenuated by melanin during its passage through the epidermis. In the dermis, collagen bundles absorb the radiation and re-emit light back towards the skin surface that can again be attenuated by melanin in the epidermis. As melanin absorbs UVR much more than visible light, UV light examination enhances the contrast between the normally pigmented areas and the hyperpigmented areas [48]. However, the quality of the black and white photographs depends on many parameters, such as photographic conditions (environmental illumination, photographic angle, development bath...), that are difficult to reproduce precisely. The Visioscan VC98[®] (C+K Electronic, Cologne, Germany) is a UV camera that has to be applied directly on the skin, avoiding the influence of environmental light. Image analysis is performed

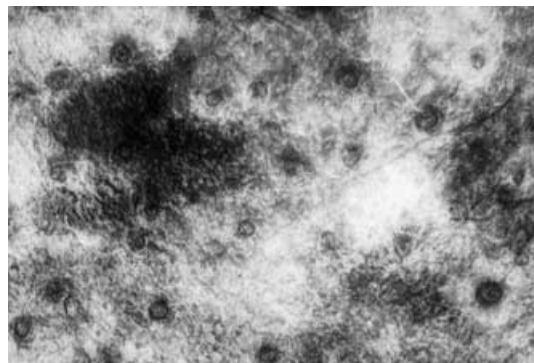


Figure 2 Uneven distribution of melanin inside the epidermis as revealed by *in vivo* examination under ultraviolet light.

on the pictures [8, 9, 49, 50]. By this UV light-enhanced visualization (ULEV) method, it is possible to record the cumulative amount of melanin present in the epidermis (Fig. 2) and to perform image analysis (Fig. 3). It must be stressed that when using the ULEV method, the discrimination is not very effective when a large amount of melanin is present in the skin, such as that encountered in phototypes V and VI following Fitzpatrick's classification [51].

Analytical methods

Image analysis

Image analysis of high-resolution digital imaging of a restricted or a wide skin area can be used to quantify the effects of skin-lightening products [2, 8, 49, 52, 53]. Such measurements can quantify the area of hyperpigmented spots with regard to the background skin colour. The reproducibility and reliability require strict calibration and appropriate controlled repositioning.

There are two major methods for evaluating hyperpigmented spots by image analysis [53]. The first one is to count the number of pixels whose brightness intensities are distributed in a certain threshold level using the histogram analysis of the frequency distribution on all pixels. The second method is to extract pigmented spots from the image and measure their total area when colour difference between a spot and its surrounding skin area exceeds a certain level.

Corneomelametry

The stratum corneum of the volunteers can be sampled by cyanoacrylate skin surface strippings in order to quantify the melanin content of corneocytes under the microscope [54]. This method is called corneomelametry when samples are stained with the Fontana–Masson method before measuring light transmittance using photodensitometry under the microscope [49].

In-use tests

When the product has demonstrated the required abilities, it is distributed to a great number of consumers to be used in normal conditions [33]. The test population must be carefully selected, in particular, according to the age, gender, sun exposure habits and nature of the pigmentary disorder. Both the consumer and the expert examiner can give their opinions about the efficacy and tolerance of the

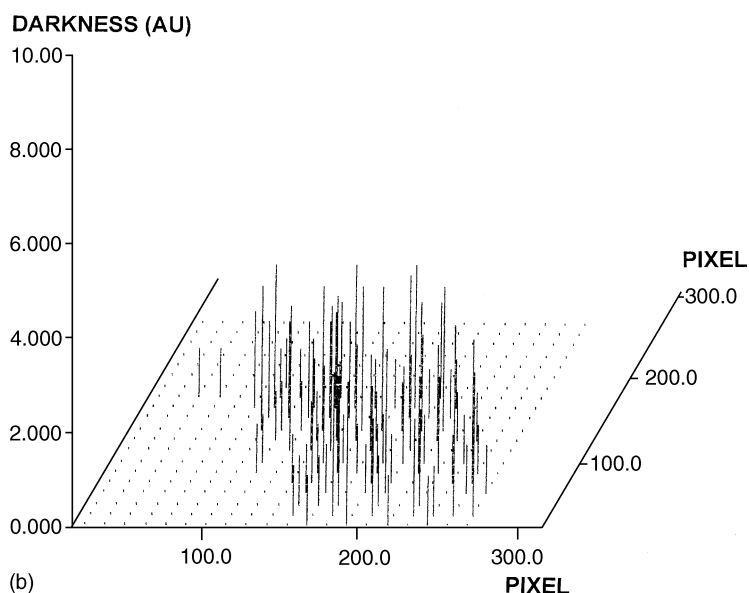
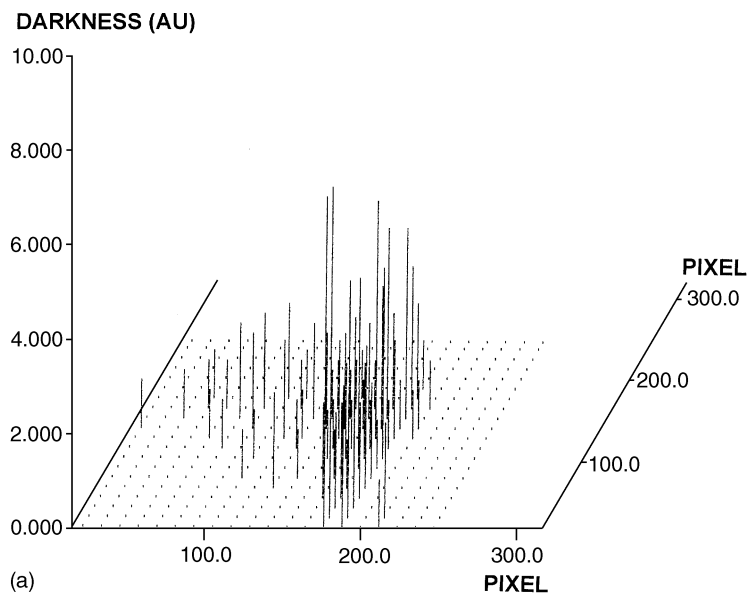


Figure 3 Image-analysis-assisted corneometry showing the decreased area (pixels) and darkness (arbitrary units, AU) of melanin pigmentation of the stratum corneum. Comparison of values before (a) and after (b) a 2-month treatment using a skin-lightening formulation.

formulation. It is not exceptional to reach large inter-individual variability among the panelists. A multi-parametric approach usually allows the development of efficacious skin-lightening products.

Skin-lightening agents

A variety of skin depigmentation formulations are commercially available. They contain one or several different active compounds. They may correspond to prescription drugs, over-the-counter products and cosmetics. Others are of uncontrolled, traditional or prohibited make. The latter flourish in countries with

less stringent regulatory requirements. There are indeed major differences in product registration requirements in various countries and markets. As a result, efficacy and safety of skin-lightening products may vary according to regions of the world.

Given the previous considerations, the skin-lightening agents can be classified following their mechanism of melanogenesis inhibition [55]. However, some agents act on several steps of the process. In addition, most of the proprietary skin-lightening formulations contain a combination of compounds in order to provide better efficacy via supplementary or synergistic actions [56–59]. Skin-peeling agents

such as trichloroacetic acid may be used to destroy and remove skin tissues loaded with melanin [15]. In all instances, sun protection measures are recommended in addition to the skin-lightening formulations.

Interest in discovery of new skin-lightening agents is currently on the increase by the cosmetic, consumer product and pharmaceutical industries. This situation corresponds to a perceived need in the market place for novel agents with increased efficacy and improved safety profiles. Thus, new compounds frequently appear in cosmetic industry trade journals. Unfortunately, statistically significant demonstrations of comparative clinical efficacy and safety of these products are not always met.

Hydroquinone

Hydroquinone is the most prescribed skin-lightening agent worldwide despite its inconsistent effects and safety concerns [11, 51, 55, 60]. This hydroxyphenolic agent was originally believed to act mainly by inhibition of tyrosinase because of its structural analogy to melanin precursors [61]. Other putative mechanisms of action include inhibition of DNA and RNA syntheses, degradation of melanosomes and destruction of melanocytes [62]. The clinical efficacy depends on the hydroquinone concentration, nature of the vehicle and stability of the formulation. Hydroquinone is often used at 1.5–5% concentrations.

Combined formulations have shown efficacy [56, 57]. A time-honoured formulation associates hydroquinone (5%) with tretinoin (0.1%) and dexamethasone (0.1%) [56]. Tretinoin acts by preventing the oxidation of hydroquinone, improving stratum corneum penetration, allowing pigment elimination and increasing keratinocyte proliferation. The corticosteroid decreases the irritative effects of hypopigmenting agents, and also inhibits melanin synthesis by decreasing cellular metabolism. This formulation has been reported to give good results in decreasing the epidermal hyperpigmentation of melasma. By contrast, it shows insignificant effect on dermal post-inflammatory pigmentation.

The depigmenting effect of hydroquinone is potentiated by buthionine sulphoximine and cystamine, as a result of the reduction of intracellular levels of glutathione and thus of the impairment of glutathione-dependent cell-detoxifying protection [63].

Hydroquinone concentrations above 5% are not advisable because of the side-effects [64]. Irritation is not infrequent. Exogenous ochronosis and pigmented colloid millium have been reported [65–69].

Permanent depigmentation may ensue during long-term treatment with high concentrations. Hydroquinone is highly reactive and is a potent melanocyte cytotoxic and mutagenic compound, and this is why hydroquinone is not authorized for use in cosmetic products anymore.

Hydroquinone monobenzyl ether (HMBE, monobenzone)

This hydroquinone derivative is metabolized by tyrosinase into reactive free radicals damaging the melanocytes permanently. A burning sensation after use is common. Its effect has not limited the site of application. It is a potent sensitizer and can cause an unpleasant confetti-like melanoleucoderma in some patients. This is why it is only indicated in case of severe vitiligo to bleach the non-affected sites [70].

Hydroquinone monomethyl ether (4-hydroxyanisole, mequinol)

This other hydroquinone derivative [71] is enzymatically oxidized in melanocytes to produce highly cytotoxic compounds such as quinones, which are responsible for the destruction of pigment cells resulting in skin depigmentation [61, 71, 72]. This compound acts synergistically with tretinoin in the treatment of hyperpigmented lesions [73–75].

Arbutin

This hydroquinone β -D-glucopyranoside from the *Uvae ursi* folium appears to inhibit tyrosinase activity [76].

Corticosteroids

The bleaching effect of topical corticosteroids is obvious [77], but the mechanism of melanogenesis inhibition is still unclear. Hydrocortisone, triamcinolone, betamethasone, fluocinolone, dexamethasone and other corticosteroids are often used in association with other skin-lightening agents to enhance their activity or to decrease their irritating effects. Treatments for long periods of time must be avoided because of the numerous adverse effects [78, 79].

Tretinoin (retinoic acid)

Tretinoin by itself acts as a weak depigmenting agent by inhibiting both constitutive and facultative

melanogenesis. It helps to disperse melanosomes within the keratinocytes. In addition, it also accelerates the turnover rate of the epidermis [80–83]. Its main adverse effect is irritation at the site of application. Post-inflammatory hyperpigmentation is also possible although rare.

It is acknowledged that tretinoin increases the depigmenting effect of hydroquinone and its derivatives. A specific mechanism could be involved [75]. In fact, retinoids behave as efficacious uncompetitive inhibitors of glutathione with tretinoin acting as the most potent retinoid capable of inhibiting this enzyme [84]. Retinoic acid is also shown to significantly suppress the expression of human glutathione as a result of decreased transcription from its gene [85].

Azelaic acid

Isolated from *Malassezia* spp., this non-toxic dicarboxylic acid is *in vitro* a competitive inhibitor of several mitochondrial oxido-reductases. It also inhibits tyrosinase and DNA synthesis. In addition, it acts as a scavenger of reactive oxygen species [86–90]. Azelaic acid can be used in combination with glycolic acid [91].

Kojic acid

Kojic acid produced by *Aspergillus* spp. and *Penicillium* spp. is an inhibitor of tyrosinase [92–94]. The product can be combined with other skin lighteners [58]. Kojic acid has recently been banned in Japan because of mutagenicity concerns.

Magnesium L-ascorbyl-2-phosphate

Vitamin C (ascorbic acid) is known as an agent that inhibits melanin formation by reducing *o*-quinone formation and reduces oxidized melanin [95]. It is an antioxidant and acts synergistically with vitamin E in several oxidation steps of melanin synthesis [96, 97]. However, vitamin C is quickly oxidized and decomposes in an aqueous solution. Therefore, magnesium-L-ascorbyl-2-phosphate (MAP) was synthesized to resolve this problem [98]. This compound releases L-ascorbic acid in the presence of skin phosphatases. However, MAP easily dissociates to anions that makes passing through the skin difficult. Therefore, iontophoresis was used to enhance MAP penetration through the skin [99].

Niacinamide

Niacinamide is the biologically active form of vitamin B3. It is supposed to act via a limitation of melanosome transfer from melanocytes to keratinocytes [100].

4-*n*-butyl resorcinol

This resorcinol derivative strongly inhibits the activities of tyrosinase and TRP1 [101].

Serine protease inhibitors

A new approach to inhibit melanization of the skin is to reduce the transfer of mature melanosomes from melanocytes into keratinocytes and is mediated by the PAR-2. Soya bean extracts offer routes to reduce melanosome transfer by this mechanism [8, 14].

Mercury

Mercury, once widely used, is a toxic compound. It persists as an ancient 'skin-bleach' product in some countries [78, 79]. The daily uptake from skin absorption may be 20 times that taken in food. Toxicity is therefore a major concern, and legislation bans its use in many countries.

α - and β -hydroxyacids

High concentrations of several α - and β -hydroxyacids including glycolic acid and *o*-hydroxybenzoic acid (salicylic acid) are used as chemical peels [102–104]. The latter compound is also a non-competitive inhibitor of tyrosinase [105]. These compounds may be used in combination with hydroquinone and kojic acid [57, 96].

Ellagic acid

Ellagic acid is a polyphenol found in a variety of plants including strawberry, geranium, eucalyptus, green tea and tara. The compound is a strong antioxidant and may suppress tyrosinase activity. It inhibits skin pigmentation resulting from UV irradiation. It is also thought to suppress melanogenesis without injuring melanocytes [106].

Resveratrol

Oxyresveratrol extracted from *Morus alba* is a highly non-competitive inhibitor of the DOPA oxidase activity

of mushroom tyrosinase [107]. It can be used as an additive compound in skin-lightening cosmetics [108].

Glabridin and liquorice extract

Oil-soluble liquorice extracts including glabridin inhibit melanogenesis [109, 110]. However, contact allergic dermatitis can develop to these compounds [111].

Linoleic acid and α -lipoic acid

Linoleic acid and α -lipoic acid have been reported to decrease the UV-induced pigmentation [31, 32, 112].

Conclusion

A variety of commercial skin-lightening formulations are available. Frequently two or more of the active compounds are used in combination therapies. Comparative clinical trials with objective and controlled assessments are not numerous. This part of skin-biology control merits further investigations to reach good standards in evidence-based cosmetology.

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