J. of Korean Oil Chemists' Soc.,
Vol. 15. No. 3, Sep, 1998. 1~12

〈總 說〉

Current status of whitening agents and their future in Japan

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(Received August, 19, 1998)

Skin whitening agents, possible methods of controlling melanogenesis, and future considerations for skin whitening are discussed with respect to active ingredients and related substances in Japan.

It has long been believed that whitening (lightening) the skin is an essential element of beauty since white skin can hide a wide variety of skin problems. Xi-shi, a rare beauty in ancient China, was said to be so white as to appear ill by present day standards. The life and culture in the deepest recesses of the court, where sunlight scarcely penetrated, may have helped to foster this belief.

In contrast is the currently held belief that the light brown pigmentation resulting from a summer suntan is somehow healthy. This is due to a vague understanding among the general populace that vitamin D is somehow synthesized through exposure to ultra-violet (UV) and to the widely-held folk belief that people with a suntan are less likely to catch a cold. Moreover, some people today are even willing to acquire a suntan artificially in order to create the appearance of 'health' which is itself considered stylish.

It is now widely recognized that UV exposure is not without consequences.

UV exposure can result in a wide range of skin problems including photoaging such as wrinkles and spots, photosensitization, and skin cancer.

Under these circumstances, the market in Japan for cosmetics containing sunscreens and skin whitening agents has rapidly expanded. In particular, the market for skin whitening products was estimated to be 15.7 billion yen in 1989, 36.5 billion yen in 1990, just after kojic acid and arbutin were commercialized, and 46.9 billion yen in 1997, when ellagic acid was commercialized. The market will surely grow in 1998 with the commercialization of rucinol. Other estimates put the market at over 100 billion yen.

This paper presents current topics and future possibilities concerning skin whitening ingredients, focusing on melanin as the key term. Among these ingredients related to oleochemistry[1], the authors will introduce some substances having a possible affect on melanogenesis, for example, antioxidants such as tocopherol and inflammatory or signal transducable lipids derived from the cell membrane such as arachidonic acid and diacylglycerols.

On the other hand, oil- and fat-based products, which are necessary and indispensable for detergents and vehicles, will not be discussed here. It goes without saying that sunscreening agents play an important albeit passive role in skin whitening because UV is perhaps the most important factor responsible for the acceleration of melanogenesis. Salicylate derivatives, benzophenons and p-aminobenzoate derivatives are well-known[2] but will not be discussed here.

For a thorough discussion about melanin and skin pigmentation, the reader is advised to refer to the many excellent texts available on these subjects[3-7].

A) Melanin and color of human skin

Among the various factors affecting the color of human skin, melanin is the most important. Melanin is synthesized from tyrosine, an amino acid, in the melanosomes of melanocytes (MC) which are found in the basement layer of the epidermis (see Figure 1).

The distribution of MC varies widely; there are many in the face and forearm and relatively few in the foot, hip and palm. It has been reported that an average of 1500 MC per mm2 exist in the skin. Melanosomes in MC are transfered (phagocytosed) into neighboring keratinocytes (KC). The color of human skin is thus determined mainly by the number and volume of melanosomes in KC rather than simply by the number of MC. This is the basis for racial differences in skin color. The color of human skin is also affected by the ratio of eumelanin (black to dark brown) to pheomelanin (light brown).

KC are eliminated as debris after about 28 days through a process called keratinization.

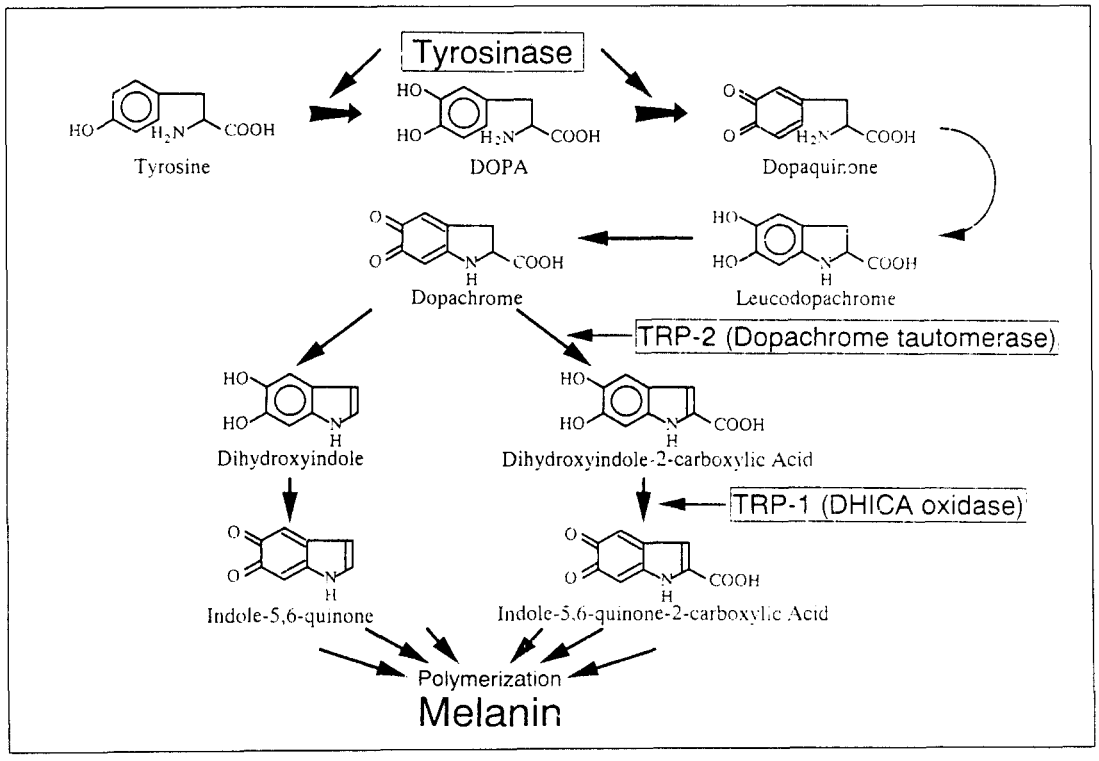

Fig. 1 Process of melanogenesis (Ref.1)

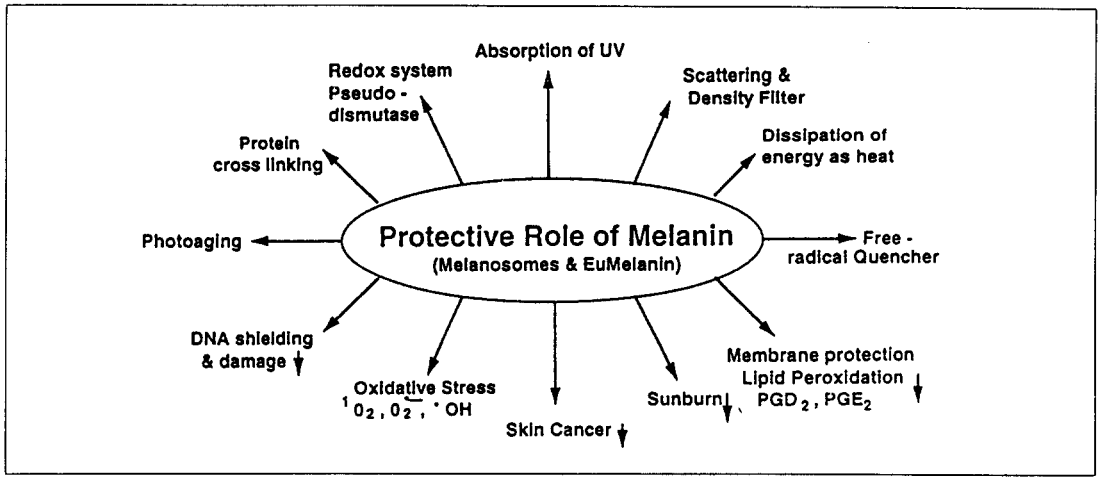

Fig. 2 Photoprotective role of melanin in human skin (Ref.8)

Melanin included in KC, as being partially degraded, is also eliminated at almost the same rate. When melanogenesis is accelerated and continued, or when the process whereby melanin is eliminated has been disrupted, skin pigmentation (commonly known as spots) occurs. The authors intend to discuss ingredients that effectively reduce melanin. It should be noted that although excessive melanin in the skin may be troublesome with respect to beauty, melanin itself is not harmful but actually protects the skin (and thus the body) from UV. It also can trap free radicals, and thus melanin also possesses anti-oxidant properties. Figure 2 shows the effects of melanin[8].

Although an excess or deficiency of melanin may be related to serious pathology, the details of such are not shown here. The authors intend to restrict the discussion to epidermal pigmentation caused by MC though melanin sometimes decends into the dermis or is produced in the dermis.

B) Suppressers of tyrosinase

Tyrosinase is the most important enzyme in melanogenesis. It catalyzes the first two steps from tyrosine to DOPA (dihydroxyphenylalanine) quinone via DOPA[9].

It is a metaloenzyme containing copper in the active center. Numerous studies have been carried out on tryosinase activity, because the suppression of tyrosinase activity is thought to be the most effective way to achieve skin whitening. In fact, all of the six active skin whitening agents approved for use in quasi-drugs in Japan, whose claim is to prevent spots and freckles caused by sun exposure, suppress tyrosinase activity in one way or another. Although numerous other ingredients are available for suppressing tyrosinase activity in addition to these approved six active ingredients, they have not always been recognized effective in humans.

Skin whitening strategies other than suppresion of tyrosinase activity are currently being considered, but it is doubtful that these strategies will continue to exert a large impact on the future of skin whitening. Rather, future trends can be expected to capitalize on the synergistic effects of tyrosinase suppression in combination with other ingredients.

Strategies for suppressing tyrosinase activity include

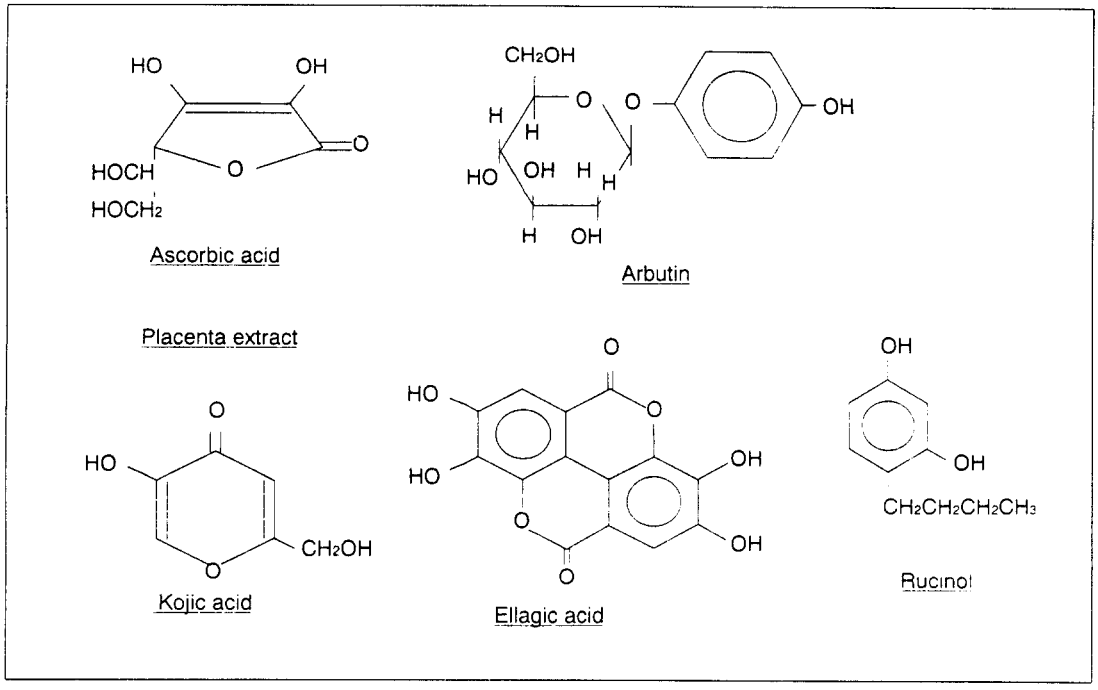

Fig. 3 Structures of skin whitening active ingredients

a) inhibition of tyrosinase activity,
b) inhibition of glycosylation
(tyrosinase is a glycoprotein),
c) suppression of tyrosinase mRNA, and d) reduction of tyrosinase protein.

1) Crude drugs

There are numerous examples of crude drugs such as extracts of mulberry and peony. In fact, research in this area has been so active[10-13] as to suggest that most plants possess some kind of tyrosinase-suppressing activity. Even common plants (such as the calyx of the puchi-tomato[14]) have been thoroughly studied. Suppression of tyrosinase activity has been also reported for exotic South American plants[15], various mushrooms[16], sea weeds[17], lichens[18] and the shells of some deep sea mollusks[19]. Moreover, countless metabolites produced by microorganisms have been examined. Four of the 6 active ingredients (Figure 3) currently approved as quasi-drugs as well as others are discussed (the other two currently-approved active ingredients are discussed later).

a) Kojic acid[20,21]

Kojic acid (KA), marketed by Sansho Seiyaku Co. Ltd. in 1989, had a major impact on the skin whitening market. KA is a-pyrone compound produced by molds such as Aspergillus sp.. KA inhibits tyrosinase activity by chelating the copper ion at its active center. KA also possesses antioxidant activity.

b) Arbutin[22,23]

Arbutin (AR) was commercialized by Shiseido Co. Ltd. in 1990. AR, the glycoside of hydroquinone, is an ingredient in cranberry and bearberry.

c) Ellagic acid[24,25]

Ellagic acid (EA) is an active ingredient developed by Lion Corp. and commercialized in 1997. EA is a polyphenol compound found mainly as the ellagitannin in a variety of plants such

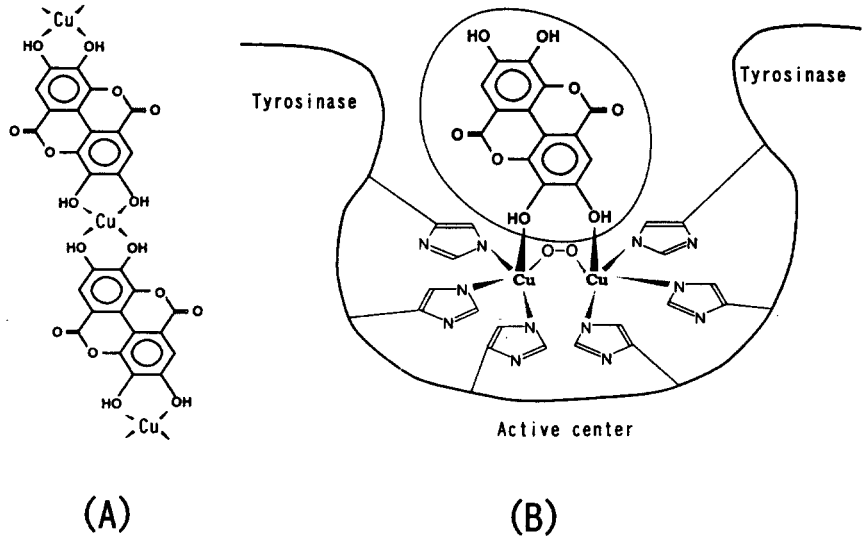

Fig. 4 Chelation to copper by ellagic acid (supposed)

as strawberry, eucalyptus, geranium and tara. EA has been approved as a food additive due to its antioxidant activity. EA is thought to inhibit tyrosinase activity by chelation of the copper ion(s) at the active center by the assumed mechanism shown in Figures 4(a) or (b).

When melanoma cells were cultured in a medium containing EA, tyrosinase activity was suppressed and the color of the cells was lightened. Moreover, the efficacy of EA is evidenced by its ability to prevent spots when applied topically on the skin of brownish guinea pigs irradiated by UV. Furthermore, the efficacy for preventing pigmentation on the human forearm caused by UV is 86%, and no side effects have been reported (Figure 5).

d) Rucinol (4-n-butyl-resorcine)[26]

Rucinol (RU) was commercialized by Pola Co. in 1998. RU, one of the derivatives of cresol, is similar to substances contained in fir trees.

e) Others

Mulberry bark extract intensely suppresses

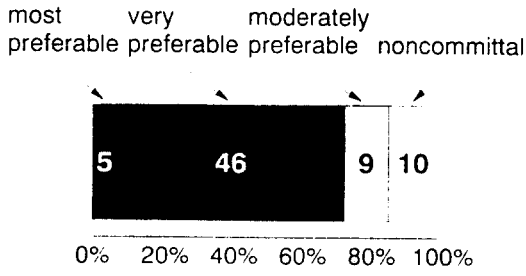

Fig. 5 Effect of ellagic acid on human skin irradiated by UV (Ref.24)

tyrosinase activity in vitro. The active ingredient of oil-soluble licorice extract was found to be glabridin [27,28]. Many crude drugs are expected to exhibit humective and/or anti-inflammatory effect as well as suppression of tyrosinase activity.

In addition, tunicamycine, produced by Streptomyces lysosuperficus, has been shown to inhibit the glycosylation of tyrosinase.

2) Hydroquinones[29-31]

Although hydroquinone is known to exhibit

skin whitening activity, it is not used generally in Japan because of its irritation potential. Use of hydroquinone monobenzylether is prohibited since it is thought to damage MC and cause white spots.

3) Fatty acids

Unsaturated fatty acids such as oleic, linoleic and -linolenic acid suppress tyrosinase activity in cells[32,33]. These are not enzyme inhibitors but rather suppressers of the biosynthesis of tyrosinase. Conversely, saturated fatty acids such as palmitic acid are known to accelerate melanogenesis.

Lactic acid, one of the -hydroxy acids, suppresses tyrosinase activity due to the suppression of gene expression[34]. It has also been reported to affect proteinkinase C.

Azelaic acid, dicarbonic acid produced by Pityrosporum orbiculare (Malassetzia furfur), was found to exhibit a skin whitening effect[35,36]. Although it improves skin pigmentation somewhat, the irritation sensation cannot be neglected.

4) Chelators[37]

Tyrosinase is a metaloenzyme containing copper atoms at its active site, so enzyme activity can be suppressed by removing copper from that site. KA and EA exhibit this chelating effect. However, chelating activity and the subsequent inhibitory effect on tyrosinase do not always occur proportionally. The time will surely come when other effective substances can be synthesized using computer aided molecular design.

C) Suppressers of tyrosinase-related proteins (TRPs)

TRP1 (5,6-dihydroxyindole-2-carbonic acid oxidase) and TRP2 (dopachrome tautomerase), which are related to melanogenesis, are also worth consideration[38,39]. Oil-soluble licorice extract is known to suppress TPR2[40].

However, it is not clear how important these enzymes are since there are no known substances that inhibit only TPRs.

D) SH (thiol) compounds

There are two pathways after DOPAquinone is catalyzed by tyrosinase: a) the pathway by which black to black-brown eumelanin is synthesized via leucoDOPAchrome and DOPAchrome, and b) the pathway by which light brown pheomelanin is synthesized from DOPAquinone and thiol compounds. Therefore, lighter-colored pheomelanin can be expected to increase in the presence of abundant thiol compounds[41,42]. Glutathion has been approved as a drug for improving skin spots (per os). When glutathion in the cell is depleted, tyrosinase activity increases[43]. Also, cystein, one of the thiol compounds, suppresses melanogenesis in the cell, but its effect on humans has not yet been reported. Because thiol compounds are easily oxidized, they can be expected to possess antioxidant activity.

E) Antioxidants[44-46]

A wide range of substances, both natural and synthesized, are known to act as antioxydants: catalase · peroxydase · superoxidedismutase (enzymatical degradation or elimination of oxidants), transferrinalbumin (stabilization of metal ions), ascorbic acidtocopherolscarotenoids (capture of radicals). Recently, polyphenols and flavonoids in red wine as well as cacao beans have attracted interest. Only ascorbic acid is discussed here.

1) Ascorbic acid (vitamin C) and its derivatives

Ascorbic acid (AA) is approved both as a drug and a quasi-drug. The action mechanism

is suppression of melanogenesis and destruction of melanin by AA's reductive activity, rather than by suppression of tyrosinase activity. Furthermore, because ascorbic acid can accelerate collagen synthesis (activation of proline hydroxylase), a skin activating effect can be also expected. Since ascorbic acid itself is unstable, a number of derivatives have been developed including L-ascorbate stearyl, ascorbate dipalmitate, and magnesium L-ascorbate phosphate[47]. These derivatives are then hydrolyzed to AA in the skin.

F) Skin activators

Activating the skin by promoting epidermal cell turnover, or by moisturizing or nourishing cells, has been demonstrated to be effective for skin-whitening. α-Hydroxy acids such as glycolic acid (referred to as 'fruit acid')[48] and humectants such as birch extract are well-known examples. Such activators are expected to exhibit a skin whitening effect when used in conjunction with other active ingredients, rather than when used independently. Only placenta extract is discussed here.

1) Placenta extract[49]

Placenta extract (PE) is approved as an active ingredient for quasi-drugs. Although PE has a slight inhibitory effect on tyrosinase, its skin whitening effect is thought to result from a skin activating effect. PE, the aqueous extract from the fresh placenta of pregnant cows, is rich in amino acids, proteins, vitamins and minerals.

G) Anti-inflammatory agents

When inflammation occurrs in the skin, numerous inflammatory substances are released[50,51] and these stimulate MC directly and/or indirectly, thus accelerating melanogenesis. Of these substances, especially plostagrandin (PG) E2 and leucotrien (LT) C4 exhibit active melanogenetic properties[52-54]. LT is also reported to be synthesized in melanoma cells[55]. Since erythema and pigmentation following UV irradiation can be effectively suppressed by (rank order) clobetasol propionat> ehydrocortisone butyrate> indomethacin[56], it is thought that inhibition of cyclooxygenase (related to PG synthesis) and/or lipoxygenase (related to LT synthesis) will be effective in suppressing melanogenesis. But, the effects of these drugs are different depending on whether the skin has been irradiated by UVA or UVB[56], so further investigation is needed about the relationship between UV irradiation, inflammation and melanogenesis.

Furthermore, phospholipase (PL) A2 , the enzyme releasing arachidonic acid, which is a precursor for PG and LT, accelerates melanogenesis[57]. Since one of the effects of steroids is inhibition of PLA2, a similar relation may exist between the two.

Histamine also stimulates MC and accelerates melanogenesis[58]. Using the analogy of KC, histamine activates adenylate cyclase through its receptor(s) and increases c-AMP content[59]. Since tyrosinase activity is suppressed by the H2 blocker[60], new suppressers of melanogenesis can be expected to be found among antihistamine agents.

H) Blockers of outer stimuli

Since some extracellular stimulus is needed to accelerate melanogenesis in MC, reducing extracellular stimuli to MC can be expected to reduce melanogenesis. Therefore, a blockade of hormones and/or cytokines can be expected.

For example, UV stimulates KC and accelerates the production of interleukin (IL) -1, which releases endothelin (ET) through the autocrine

mode. ET stimulates MC to increase Ca content in the cytoplasm, thus accelerating melanogenesis[61,62]. Chamomile extract is reported to block the effect of ET[63]. Other factors accelerating melanogenesis include cytokines[64,65] such as b-FGF and EGF (especially, b-FGF also exhibits active proliferative activity on MC) and hormones[66,67] such as MSH and ACTH (peptide hormones) or estradiol (steroid hormone). If these factors act through specific receptors, then an effective strategy will be elucidating the mechanism affecting the number and/or activity of receptors and searching for blockers related to these sites.

l) Regulators of intracellular signal transduction

A number of signal transduction mechanisms are already known to affect melanogenesis. These include ; 1) c-AMP protein kinase (PK) A, 2) diacylglycerol (DG)PKC, 3) inositolphosphatesCa, 4) c-kit, c-mettyrosine kinase. Therefore, melanogenesis can be affected by the inhibitors of these systems. For example, H89, the PKA inhibitor, whitens the melanoma cell, and DG, the PKC activator, causes skin pigmentation in the brownish guinea pig[68]. However, these influences are very complex, considering that the various action mechanisms are not independent and that extensive cross-talk exits in the systems, and that there are virtually no

Table. 1 in vitro Evaluation methods of candidates for skin whitening agents

Item	Index
Suppresers of tyrosinase	Tyrosinase activity ·enzyme (mushroom, cell extracts) ·cell (melanoma, melanocyte)
Suppressers of TRP	TRP1 and/or TRP2 activity ·enzyme (cell extracts)
SH (Thiol) compounds	Ratio of eumelanin and pheomelanin ·cell (melanoma)
Antioxidants	Content of peroxy lipids or radicals Superoxidedismutase (like) activity
Skin activators	Proliferative activity Oxidoreductive activity to pigment ·cell (keratinocyte, fibroblast)
Antiinflammatory agents	Cyclooxygenase and/or lipoxygenase activity Content of PG and/or LT ·enzyme (extracts of testis, platelet etc.) ·cell (fibroblast) Histamine release ·cell (mast cell)
Blokers of outer stimuli Regulators of signal transduction	Content of cytokines Number of receptor Targeting enzyme activity

known substances that manifest only a single reaction mechanism.

In addition, DNA damaging agents such as 4-NQO and MMS produce skin pigmentation in the brownish guinea pig[69] although the action mechanism is not clear. Cell adhesion molecules such as integrins on the cell surface also seem to be related to melanogenesis[70].

J) Others

Increasing knowledge about the genetic basis of enzymatic action on melanogenesis can be expected to provide a means of treating black and blue nevi as well as controlling skin whitening.

Besides, retinoic acid, known as an anti(skin)aging agent, is reported to be useful for hyperpigmented lesions[71], but its action mechanism remains unclear.

K) Evaluation of skin-whitening active ingredients

A number of methods are available for evaluating skin whitening active ingredients. The main trends in the development of skin whitening agents are, in turn, of in vitro (enzymes, cells)in vivo (animals)human. Generally, depending on the specific ingredient, the evaluation should be first performed using an in vitro system including cells, and next, the selected substances should be evaluated by an in vivo system using an animal model. Finally, candidates would be applied to humans after confirmation of safety. The in vitro evaluation methods for the items mentioned in the present paper are shown in

Table 2 Evaluating methods for preventing or improving skin pigmentation

	Materials	Methods
Enzymes	Tyrosinase ·mushroom ·cell extracts Enzymes other than tyrosinase ·DOPAchrome tautomerase ·Other enzymes based on each hipothesis (SOD, catalase etc.)	Activity assessment ·Absorbance (DOPAchrome produced from DOPA or Tyrosine) ·Radioactivity (^3H_2O produced from ^{3}H-Tyrosine) Melanin content ·Radioactivity (^{14}C-Melanin produced from ^{14}C-Tyrosine) Activity assessment ·HPLC (DHICA produced from DOPAchrome) (each assay method)
Cells	B16 melanoma Melanocyte (human, mouse) ·Tyrosinase ·activity ·protein ·mRNA ·Melanin content ·Cell color ·Other markers ·proliferation ·appearance ·inner cell Melanocyte ·Cell activity (shape, dendrite) Melanocyte-keratinocyte Co-culture system ·Melanin metabolism	 Radioactivity (^3H_2O produced from ^{3}H-Tyrosine) Western blotting Northern blotting Absorbance (after solubilization) Radioactivity (^{14}C-Melanin produced from ^{14}C-Tyrosine) HPLC (fractional analysis of eumelanin and pheomelanin) Macroscopic analysis (Photograph) Cell blotting and image analysis Cell number, DNA, Protein Microscopic analysis with / without staining Electron microscopic analysis Microscopic analysis (electron) Microscopic analysis
Animal	Brownish guinea pig ·preventing or improving effect to skin pigmentation caused by UV irradiation	Macroscopic analysis (Photograph) Chromametry (Chromameter, Mexameter etc.) Histochemistry (electron) Microscopic analysis of peeled epidermis or cut skin stained by DOPA / premelanosome staining
Human	Human ·preventing or improving effect to skin pigmentation caused by UV irradiation ·preventing or improving effect to melasma, freckle etc.	Macroscopic analysis (photograph) Chromametry (Chromameter, Mexameter etc.)

Table1, and the more general methods using tyrosinase or melanoma are shown in Table2. Although the evaluation method will differ depending on the type of substance being evaluated, the (black) color index of melanoma cells is generally accepted as a comprehensive and simple method for evaluating melanin content. It should be noted that the recently reported cell blotting method is very useful because it is simple, applicable to small samples, and provides a quantitative result[72]. According to the authors' experience, a large number of substances with positive results can be found. However, it may subsequently be difficult to confirm the whitening effects of these substances at the animal test stage, primarily due to the poor cutaneous absorption characteristics of these substances. Furthermore, there are many difficulties involved in commercializing these substances such as the need to conduct evaluations of efficacy and human safety, technical issues related to the stabilization of ingredients, problems in obtaining an adequate supply of substances as well as the cost.

The present paper has discussed six active ingredients currently approved as quasi-drugs for skin-whitening, other ingredients that are expected to be somewhat effective, several interesting substances which have not yet been commercialized and possible research directions for the future. Furthermore, the importance of tyrosinase is certain to remain central in the effort to develop quicker-acting and more powerful active ingredients, However, as there are several possible pathways involved in spot formation, it is very important to determine how tyrosinase suppressers can be combined with blocking agents for these pathways as well as ways to increase percutaneous absorption of active ingredients.

On the other hand, intracellular signal transduction systems are so complex that unexpected effects occur as a result of cross-talk and predicted effects sometimes fail to occur. For example, inhibitors of PKA also inhibit PKC to a greater or lesser extent, and calmodulin affects not only adenylate cyclase but also phosphodiesterase. In addition, because MC can effect KC through the melanosomes phagocytosed by KC[73], and because ceramides, regarded as very important from the viewpoint of skin roughness, can cause apoptosis[74], it is necessary to consider melanin metabolism under all circumstances throughout the skin. Furthermore, progress in genetic analyses are expected to result in the development of new skin whitening agents.

Advances in research concerning melanin metabolism and new active ingredients identified in these studies can be expected to result in the development of new and highly effective products.

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Both forearms of seventy male volunteers were irradiated by UV at one MED once a day for three days. The active sample (cream with 0.5 % ellagic acid) and the placebo (cream without ellagic acid) were applied topically there three times a day for six weeks. Efficacy was evaluated according to the double-blind controlled study.