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Topical Retinoids

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OVERVIEW

The retinoids are a diverse class of pharmacological compounds, consisting of vitamin A (retinol) and its naturally occurring and synthetic derivatives, which possess biological vitamin A activity (Tables 1 and 2). Vitamin A generically encompasses retinol (vitamin A alcohol), retinal (vitamin A aldehyde), and retinoic acid (vitamin A acid) (Fig. 1). In clinical use, retinoids have established their effectiveness in treating acneiform eruptions (e.g., isotretinoin), disorders of keratinization, such as psoriasis (e.g., acitretin), as well as some neoplastic processes (e.g., tretinoin for leukemia, isotretinoin for squamous cell carcinomas). Additional retinoids are currently being investigated, as are novel uses of retinoids already established in clinical practice. The main focus of retinoid usage in cosmeceuticals has been its role as the mythical ''fountain of youth'' (i.e., reversal of photoaging) (Table 3). Retinoids, like all drugs, have adverse effects, the most infamous one being teratogenicity. Over 2000 derivatives have been developed in the hope of finding retinoids with increased therapeutic efficacy coupled with diminished local and systemic toxicity. The recent focus of retinoids has been on topical delivery systems, as this route not only provides a safer adverse effect profile, but also delivers a higher dose to a targeted area (i.e., the skin).

Table 1 Classification of Retinoids

Generation	Retinoid
First generation	Tretinoin (All-trans-retinoic acid)
	Isotretinoin (13-*cis*-retinoic acid)
Second generation	Etretinate (Ro 10-9359)
	Etretin (Ro 10-1670)
Third generation	Arotinoid (Ro 15-0778)
	Arotinoid ethylester (Ro 13-6298)
	Arotinoid methyl sulfone (Ro 14-9706)
	Adapalene (CD271)
Naturally occurring in humans	Retinol (vitamin A)
	Retinal (vitamin A- aldehyde)
	Retinoic Acid

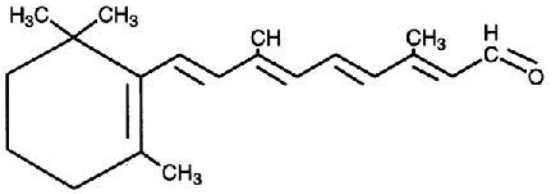
This chapter provides a review of topical retinoids, focusing on the potential cosmeceutical applications of this class of drug. Oral retinoids with no significant cosmeceutical activity, such as acitretin, will not be covered. Note that the definition of drug versus cosmeceutical for this class is regulatory (man made) and not biological.

HISTORICAL BACKGROUND

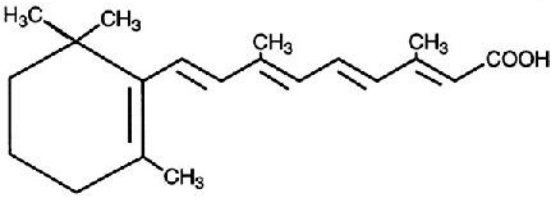
The ancient Egyptians recognized the importance of vitamin A activity as early as 1500 BC, as evidenced by early writings in ''Eber's Papyrus'' describing the benefits of liver in treating night blindness (1). However, it was not until the early twentieth century that definitive knowledge of this substance was discovered. In

Table 2 The Roles of Naturally Existing Retinoids

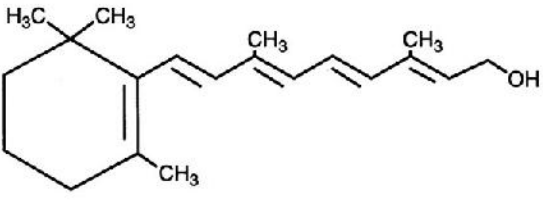
Retinoid	Role
Retinol	Growth promotion
	Differentiation/maintenance of epithelia
	Reproduction
Retinal	Vision
Retinoic acid	Growth promotion
	Differentiation/maintenance of epithelia



all- trans retinal



all- trans retinoic acid



all- trans retinol

Figure 1 Structure of retinoids.

1909, a fat-soluble extract from egg yolk was found to be essential for life (2). This substance, initially termed ''fat-soluble A'' (3) and later named ''vitamin A'' (4), was also found in butter fat and fish oils, demonstrating growth-promoting activity (5). Synthesis of vitamin A was achieved in the 1940s and from then on an upsurge of interest in the therapeutic uses of vitamin A became apparent.

Topical tretinoin was first used successfully by Stüttgen to treat disorders of epidermal keratinization in the 1960s (6). However, the irritation produced by the concentrations and formulations used in these studies inhibited widespread acceptance. Subsequently, Kligman proved the therapeutic efficacy of topical tretinoin in acne vulgaris (7), and went on to pioneer and popularize the use of retinoids in cosmetic dermatology by demonstrating its effects on photoaged skin (8).

Table 3 Uses of Topical Retinoids

Retinoid	Proprietary name	Uses	
Tretinoin	Retin-A	Acne vulgaris	Primary
(All-trans-retinoic acid)	Renova	Photoaging	indication
		Actinic keratoses	
		Lichen planus	Secondary
		Melasma	indication
		Postinflammatory hyperpigmentation	
Isotretinoin		Acne vulgaris	
Alitretinoin (9-*cis*-retinoic acid)	Panretin	Kaposi's sarcoma	
Retinol		Cosmetic ingredient	
Retinyl palmitate		Cosmetic ingredient	
Retinyl aldehyde		Cosmetic ingredient	
Adapalene		Acne vulgaris	
Tazarotene		Psoriasis	
		Acne vulgaris	
Motretinide		Acne vulgaris	

COSMECEUTICALS

The major forms of retinoids that may be of significant interest to the cosmeceutical industry are retinol, retinal, and possibly, retinoic acid. The main role of retinoids in cosmeceuticals are in extrinsic aging (photoaging). Currently, topical retinoic acid is FDA-approved for the treatment of acne, and in the adjunct treatment of fine skin wrinkling, skin roughness, and hyperpigmentation due to photoaging, as well as reducing the number of senile lentigines (liver spots) (9–11). At present, retinol is becoming an increasingly utilized ingredient in cosmetic preparations, such as moisturizers and hair products. One reason for this is that retinol is a nonprescription preparation. It has also been demonstrated to be less irritating topically than retinoic acid (12), which makes retinol a more favorable cosmetic ingredient than retinoic acid. It is therefore necessary to review the scientific basis for use of retinoids and their purported efficacy.

RETINOL

Vitamin A is a necessary dietary nutrient, required for growth and bone development, vision, reproduction, and the integrity of mucosal and epithelial surfaces.

Vitamin A deficiency results in visual problems, such as xerophthalmia and nyctalopia (night blindness), hyperkeratosis of the skin, epithelial metaplasia of the mucous membranes, and decreased resistance to infections. Vitamin A is fat soluble, and occurs as various stereoisomers. Retinol (vitamin A1) is present in esterified form in dairy products, meat, liver, kidney, and oily saltwater fish.

For clinical purposes, vitamin A is available as retinol (vitamin A alcohol) or esters of retinol formed from edible fatty acids, primarily acetic and palmitica acid.

PENETRATION, ABSORPTION, AND CUTANEOUS METABOLISM OF TOPICAL RETINOIDS

Any active substance administered to the skin must penetrate the skin in sufficient amounts in order to have a pharmacological effect. This section presents evidence that the topical retinoids can be utilized effectively. Several methods have been utilized, including enzyme induction as a marker of effective penetration, radiolabeling, and HPLC.

Duell et al. (13) studied the penetration characteristics of all-trans-retinol (ROL), all-trans-retinoic acid (RA), all-trans-retinaldehyde (RAL), and retinyl palmitate (ROL palm) in human skin in vivo. An enzyme marker was utilized to demonstrate that penetration had occurred and to measure the potency of each retinoid. As the enzyme, cytochrome P-450-dependent RA 4-hydoxylase, is induced by retinoic acid, its induction can identify whether sufficient ROL, RAL, and ROL palm penetration and metabolism to RA occur. Therefore, this enzyme can qualitatively reflect penetration and potency in the epidermis.

Utilizing microsomal preparations from human skin biopsies, a significant induction in this enzyme was noted following topical application to human skin in vivo. After 48 h of occlusion, ROL (0.025% and greater) increased the enzyme activity significantly; however, lower concentrations did not cause significant induction. The increase in enzyme induction was nonlinear, with the higher doses only causing a small increase in activity.

RAL also caused a significant induction of enzyme activity after 48 h of occlusive application. Similarly to ROL, induction was seen at concentrations greater than 0.025%, but not lower. Enzyme activity increased in a dose-related manner, with similar peak activity to equivalent concentrations of ROL. At lower doses (0.01% and 0.025%), RAL was a greater inducer than ROL, but at higher concentrations (0.05%, 0.25%, 0.5%, 1%), ROL and RAL were equally effective inducers.

RA itself was a more potent inducer of the hydroxylase enzyme than ROL and RAL. Induction was seen in RA after 24 h of occlusion, compared to 48 h for ROL and RAL, and the degree of induction was much greater.

ROL palm applied under occlusion also induced enzyme activity. 0.6% ROL palm significantly induced the enzyme, while lower concentrations, vehicle, or equivalent concentrations of palmitate alone did not. However, ROL palm was applied for 4 days, in contrast to the 24- and 48-h studies outlined above (Table 4).

The effect of occlusion on the ability of ROL, RA, and ROL palm was also assessed. While unoccluded RA significantly induced RA-hydroxylase, a significantly greater induction occurred under occlusion. A similar effect was seen for ROL palm. However, this occlusive effect was not seen with ROL: both occluded and unoccluded sites produced a similar significant increase in enzyme induction compared to vehicle. Enzyme activity induced by 0.25% ROL (either unoccluded or occluded) was similar to that induced by 0.025% RA (under occlusion).

Whether the induction of this or other enzyme markers in the skin reflects the ability of retinoids to produce a pharmacological effect is not clear. However, cosmetic-type preparations mandate sufficient retinoid concentrations to allow adequate penetration for a pharmacological effect. As a threshold level could be identified for enzyme induction in the above study, there may also be a threshold for a pharmacological effect. An insufficient concentration in the cosmetic, or inadequate application by the consumer, may render the formulation relatively ineffective.

Cellular Uptake of Retinol

In addition to sufficient delivery of the retinoid to the skin, the retinoid should be delivered in the correct form to allow cellular uptake and metabolism. Retinoids occur in human plasma bound to proteins: retinoic acid is bound to albumin and ROL to retinol-binding protein (RBP) (14). Therefore, the possibility that

Table 4 Assaying Retinoid Effects Utilizing Cutaneous Markers

Compound	Marker	Time to induction (under occlusion)	Minimum inducing concentration (%)	Does occlusion enhance induction?
ROL	cP450-OHase	48 h	0.025	No
RA	cP450-OHase	24 h	0.001	Yes
ROL palm	cP450-OHase	4 days	0.6	Yes
RAL	cP450-OHase	48 h	0.025	N/A

Source: Ref. 13.

protein binding can determine the ability of a cell to take up retinoids has been considered. (The influence of protein binding on metabolism is discussed later.)

Hodam and Creek (15) studied the uptake of retinol, either free (in ethanol) or bound to RBP, in cultured human keratinocytes. Utilizing radiolabeled compounds, they demonstrated that the retinol uptake was much greater in the free than in the bound form. Free retinol added to the culture medium had a maximum uptake of 35% of the applied dose within 3 h of incubation, falling to 20% by 12 h. In contrast, RBP retinol had a peak uptake of 7.5% of the applied dose, detected at 24 h. Therefore, keratinocytes demonstrated a much slower uptake of RBP retinol compared to free retinol.

Cutaneous Metabolism

The metabolism of vitamin A and its derivatives in the skin is considered important to the understanding of their pharmacological effect. It has been hypothesized that the effects of ROL and RAL may result from their cutaneous metabolism to RA. While some investigators have shown that this metabolism may occur, pharmacological effects have also been seen in the absence of measurable RA. This section discusses the evidence that RA is an essential metabolite in the activity of ROL and RAL.

In vitro, metabolism of ROL, RAL, and RA was studied utilizing human skin and dermal fibroblasts (16). Radiolabeled ROL, RAL, and RA were applied either topically to the skin biopsies or to the culture media of the fibroblast suspensions and the metabolites were identified by HPLC after 24 h of incubation. The skin cultures demonstrated a gradient distribution of the retinoids within the skin: 75% of absorbed activity was in the epidermis, 20% in the dermis, and 2 to 6% in the culture medium for the three retinoids tested. Of the epidermal extracts, 60% of applied ROL remained unmetabolized. The main ROL metabolites in the epidermis were retinyl esters (18.5%), a finding that has also been demonstrated in keratinocyte cultures. RA (2%), RAL (1.6%), 13-*cis*-retinoic acid (1%), and polar compounds were also found. The dermis yielded similar metabolites, but a higher proportion of polar compounds.

RAL was also metabolized in the epidermis: 43% of the absorbed radioactivity was RAL, 9% retinyl esters, 14% ROL, and 0.8% RA. When RA itself was applied, 66% of the epidermal radioactivity was from RA, 17% from 13-*cis*-RA, and 10% from polar compounds. RA was not metabolized to ROL or RAL. Dermal fibroblasts also metabolized ROL, RAL, and RA in culture medium, but the significance of this in vivo is not yet clear. It is possible that these cells may contribute to the role of the dermis in the kinetics and dynamics of these substances.

These skin studies demonstrate the capacity for topical ROL, RAL, and RA to penetrate the skin in a gradient manner from the epidermis to the dermis.

The activity in the epidermis was five times greater than in the dermis, suggesting an accumulation of compounds in that layer. While a proportion of the absorbed compound remains unchanged within the skin, significant metabolism was seen. ROL and RAL were metabolized to RA, which may play a role in the pharmacology of these substances.

Randolph and Simon (17) utilized ROL and RA bound to their endogenous binding proteins in their in vitro study: retinol was bound to retinol-binding protein and retinoic acid was bound to albumin, as has been found in human plasma (14,17a). Dermal fibroblasts, cultured either in collagen gel or on plastic dishes, were exposed to radiolabeled ROL or RA, and metabolites were detected by HPLC. In contrast to Bailey et al. (16), ROL was not metabolized by the dermal fibroblasts, although their findings for RA metabolism did concur. This may have been because of decreased availability of ROL to the dermal fibroblasts because of its protein binding. It was therefore suggested that the metabolism of ROL might only occur under pharmacological conditions. Supporting this explanation are the findings of Hodam and Creek (15) described previously, which demonstrate decreased uptake of RBP retinol compared to free ROL in cultured keratinocytes. However, the role of protein binding in uptake of ROL in dermal fibroblasts requires further elucidation.

As certain cell types preferentially metabolize different forms of retinoids, the cell content of a tissue may influence the availability of retinol and its metabolites to the surrounding tissue. The significance of this finding in cosmetic use is not yet clear. Hodam and Creek (15), in addition to determining the effect of protein binding on cellular uptake of retinoids, also considered whether protein binding affected the cellular metabolism of the retinoids once intracellular. In both cases, retinyl esters were the major metabolite and the percentage of ROL cell-associated radioactivity that was converted to retinyl esters was independent of the mode of delivery.

Several studies have therefore demonstrated a metabolic capacity for topical ROL and RAL. Retinyl esters appear to be the major metabolite, while the formation of RA from these substances constitutes a small proportion of the metabolites formed. However, this conversion may be sufficient for pharmacological activity. In vivo studies may better quantify both metabolism and dose-response relationships.

Pharmacological Effects of Retinol In Vitro and In Vivo

In vitro and in vivo studies of retinol and its derivatives have demonstrated several pharmacological effects on the skin. However, whether these effects are caused by RA as a derivative of ROL or RAL applied to the skin is not clear. The evidence is discussed below.

Kang et al. (12) found that epidermal changes could be demonstrated in vivo following topical application of ROL, without measurable retinoic acid lev-

els. This suggests that ROL itself is active in the skin. Following 4 days of occlusive application of ROL, epidermal thickness increased significantly compared to vehicle control. This increase was dose dependent: a significant increase was seen with 0.05% ROL, and the maximum concentration used (1.6% ROL) caused an increase similar in magnitude to 0.025% RA applied over the same period. Further evidence of the pharmacological activity of ROL in the epidermis was an increase in the number of mitotic figures and in epidermal spongiosis (ranked on an ordinal scale).

Interestingly, the authors were not able to detect RA, or found only trace amounts, in the time points tested (0 to 96 h). Reverse-phase high-pressure liquid chromatography (HPLC) yielded ROL, 13-*cis*-ROL, and retinyl esters (RE) in the samples, which had been tape stripped to remove the stratum corneum prior to biopsy. These results differ from those presented above where RA was found in vitro utilizing human skin. Nevertheless, cellular retinoic acid binding protein (CRABP-II) mRNA was increased, indicating CRABP-II gene activation, which supports the idea of ROL conversion to RA. The same laboratory also demonstrated an increase in a retinoic-specific hydroxylase enzyme in vivo in a previous study (REFS). However, it is possible that ROL may indirectly mediate CRABP-II gene expression by an unidentified mechanism, other than conversion to RA.

Goffin et al. (18) compared a retinol cream to a vitamin E preparation on humans in vivo utilizing bioengineering methods. In this crossover study, subjects were exposed to environmental insults, such as ultraviolet (UV) irradiation and a topical surfactant (sodium lauryl sulfate), and assessed utilizing squamometry, corneosurfametry, and optical profilometry. The authors suggest that the retinol preparation may provide some beneficial effects against these insults and also reduce the trend in shallow wrinkling induced by the irradiation. However, these data are difficult to interpret because of the crossover study design, and also because the retinol preparation was a complex cosmetic formulation. Therefore, the effects seen cannot be attributed to the effect of retinol alone. Additionally, no vehicle control was utilized.

TRETINOIN THERAPY IN PHOTOAGING

Chronic exposure to sunlight causes a characteristic collection of signs presumed to be due to aging in the past, but are now recognized primarily as the consequences of solar and other environmental injury. This is termed photoaging or dermatoheliosis. The familiar stigmata of photoaged skin are rough, leathery skin with coarse wrinkles and yellow or mottled complexion. Histologically, the dermis exhibits changes known as solar elastosis; the collagenous connective tissue in the upper dermis is replaced by fragmented, disorganized elastic fibers (19).

Ultraviolet radiation stimulates collagenases (UV-responsive matrix metalloproteinases), thereby enhancing collagen degradation and resulting in this deficiency of dermal collagen (20). Irregular epidermal thickening is seen in photoaged skin, sometimes accompanied by irregularities in cell and nuclear size, shape, and staining reactions. Melanocytic hyperplasia is a frequent feature in chronically sun-exposed skin, seen diffusely as a background of increased pigmentation, or focally as ''senile lentigines'' (21). A telangiectatic network is often seen in photodamaged skin as the disorganized dermis fails to support vessel walls, allowing them to dilate passively (22).

Topical tretinoin (all-trans-retinoic acid), used for the past two decades as antiacne therapy, has also been found effective in the treatment of photoaging. Its role in photoaging was first described and subsequently popularized by Kligman (23). He observed that women treated with tretinoin described smoother skin with less wrinkles. This clinical observation prompted him to perform clinical trials comparing the effects of tretinoin on photoaging to an inert cream. In the first of these studies, 0.05% tretinoin in a cream base was applied twice daily for 3 months on dorsal forearms of elderly volunteers, and the results compared with similar application of an inert cream to the opposite forearms. Punch biopsy specimens, taken before and after treatment, were examined using light and electron microscopy. Skin bioengineering data were also obtained. In the second study, 0.05% tretinoin cream was applied to photodamaged facial skin and specimens obtained and analyzed in a similar fashion. A third, uncontrolled study consisted of long-term facial application of 0.05% or 0.1% tretinoin cream in over 400 healthy females. The studies demonstrated significant beneficial effects on photodamaged skin, including reversal of epidermal atropy, dysplasia, and atypia, eradication of microscopic actinic keratoses, uniform dispersion of melanin granules, new collagen formation in the papillary dermis, and angiogenesis (8). Kligman reinforced this work with animal studies using the photodamaged hairless mouse model (23).

These results were consolidated by Weiss et al. (24), who similarly demonstrated in a 4-month randomized, blinded, vehicle-controlled study that 0.1% tretinoin improved photodamaged skin, both histologically and ultrastructurally. Volunteers in the tretinoin-treated group showed significant reduction in lentigines, epidermal thickening, compaction of the stratum corneum with presence of glycosaminoglycan-like substance, increased mitoses in keratinocytes, and increased number 3 of anchoring fibrils at the dermoepidermal junction. Ellis and Weiss (9) then extended the tretinoin therapy in an open-label trial, utilizing the same subjects for up to 22 months, indicating that clinical improvement was sustained during long-term tretinoin therapy. They found that 71% of discrete lentigines had disappeared after this prolonged period. Further, the problems of dryness, erythema, and flaking of the skin associated with retinoid use had

diminished or declined after the 22-month period, with maintenance of clinical benefit.

The findings in these earlier studies have now been reinforced by a solid background of formal clinical trials (25–27). [Tretinoin reverses photoaging by epidermal and dermal effects. The epidermal effects include epidermal thinning, reduction in corneocyte adhesion, decreased melanin production, and increased Langerhans cells. The dermal effects include increased collagen production, increased angiogenesis, and decreased collagenase and glycosaminoglycans (24).]

More recently, the emphasis on research in tretinoin has branched out, for instance, fine-tuning the optimum conditions for tretinoin therapy and new uses. In a recent double-blinded, vehicle-controlled comparison of 0.1% and 0.025% tretinoin creams in patients with photoaged skin, tretinoin 0.025% showed similar efficacy to 0.1%, while showing significantly less irritation.

Having more than proved its efficacy in the reversal of photoaging, the logical question is: Can retinoid therapy also improve intrinsically aged skin? The answer to this may be on the horizon. Varani et al. (28) completed an in vitro study utilizing cell culture techniques to investigate the effects of tretinoin on skin. Retinoic acid stimulated growth of keratinocytes and fibroblasts and stimulated extracellular matrix production by fibroblasts. Adult skin from sun-exposed and sun-protected sites responded equally well, whereas neonatal skin responded minimally. The implications are that retinoids may be able to repair intrinsically aged skin as well as photoaged skin, and that retinoids may modulate skin cell function in a manner that is age-related, not simply a response to photodamage.

TOXICITY

The adverse effects of retinoids are legion, and are mostly associated with hypervitaminosis A (acute or chronic). Fetal malformations, spontaneous abortions, hyperlipidemia (particularly elevated triglycerides), bone abnormalities, skin and mucosal dryness, retinoid dermatitis, pruritus, hair loss, pseudotumor cerebri, arthralgias, myalgias, and abnormal liver function tests (increased liver transaminases and alkaline phosphatase) are among the myriad potential adverse effects of retinoid therapy (29). Most of the above effects are reversible upon discontinuation of the retinoid, although some serious effects, such as fetal malformations and bone abnormalities, are not. We do not have sufficient case population data to be certain of cause and effect and no true double-blind studies exist. Recently, two classes of nuclear receptors, the RARs (retinoic acid receptors) and the RXRs (retinoid x receptors) have been identified, which are thought to play an important

role in mediating retinoid-induced toxicity. The details of this mechanism are beyond the scope of this chapter and the reader is directed toward a recent review for elucidation (30).

Topical application has the benefit of a significantly better adverse effect profile. The most common sequelae are mucocutaneous effects, characterized by skin and mucosal dryness (xerosis, cheilitis, conjunctivitis), desquamation, erythema, and pruritus. These effects typically start after several days of therapy, peak within the first few weeks, then wane as tolerance develops (31). They are easily treatable—frequent application of emollients and other precautionary measures (such as avoidance of harsh soaps, astringents, abrasives, and excessive bathing) will ameliorate the situation. The mucocutaneous effects are dose dependent and reversible upon discontinuation of the retinoid.

Teratogenicity, well documented as the most serious side effect of oral retinoids (32), is logically the potential concern with topical retinoids. With oral retinoids, most aromatic retinoids cross the placenta; in utero exposure results in limb and craniofacial deformities, as well as cardiovascular and central nervous system abnormalities. Systemic absorption of topical retinoids, however, is thought to be negligible (33). A large retrospective study of birth defects in offspring born to mothers exposed to topical tretinoin (all-trans-retinoic acid) during pregnancy has demonstrated no significant risk (34). Animal studies by Willhite et al. (35) support these data, suggesting that the drug would not be expected to cross the placenta unless present at extremely high concentrations. Even in light of this evidence, many clinicians feel strongly about avoiding topical retinoids in pregnancy (36).

Reports of enhanced photocarcinogenicity in experimental mice exist (37), but no evidence exists of a comparable process with humans (38). Conversely, topical retinoids appear to have a protective effect against ultraviolet-induced premalignant and malignant lesions. However, skin treated with topical retinoids is more reactive to chemical and physical stresses (including ultraviolet light), because of the thinner horny layer and amplified vasculature. The concomitant use of sunscreens is therefore a necessary precaution.

THE FUTURE

Retinoids have revolutionized dermatological and cosmeceutical therapeutics for the past 2 decades. The successful trials of topical tretinoin have inspired the pursuit of other topical retinoids that could be effective in photoaging with fewer adverse effects. Undoubtedly, newer derivatives with safer adverse effect profiles will be forthcoming. Specifically, two new retinoids, adapalene and tazarotene,

licensed for the treatment of acne and psoriasis, respectively, will almost certainly be investigated for photodamage.

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10
Depigmentation Agents

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INTRODUCTION

There are a variety of facial pigmentary disorders, as listed in Table 1. Among such diseases, malignant tumors should be diagnosed and treated properly because some of them are quick to develop, destructive, or fatal. Hyperpigmentation of the face of middle-aged women, is most common; however, it is benign, and, if diagnosed and treated early, it can be prevented in the future.

Melasma is commonly observed among middle-aged women (average age of 43) (1) and is rare in men. It is a diffuse or well-circumscribed noninflammatory brown hyperpigmentation that frequently occurs around the eyes, mouth, cheeks, and forehead.

Subjective symptoms such as itching or irritation are lacking (2). Melasma is present in middle age, but is rare in women over the age of 70. An experienced old Japanese dermatologist in Kyoto City often told melasma patients, ''You need not treat melasma. Just live until the age of 70 and then the melasma you suffer from will disappear.''

The main cause of melasma is considered to be an increase in progesterone (P4) in the serum at luteal phases. Sato (1) measured various hormones by tritium (3H) radioimmunoassay in two groups of age-matched middle-aged women (average age 43) with and without melasma on the seventh days of the ovarial and

Table 1 Pigmentary Skin Disorders of the Face

I. Acquired
1. Melasma (chloasma)
2. Solar lentigo
3. Pigmented cosmetic dermatitis
4. Sun tanning
5. Tattoo
6. Ochronosis
7. Pigmentation due to atopic dermatitis
8. Phototoxic hyperpigmentation (Berloque dermatitis)
9. Posttraumatic hyperpigmentation
10. Others (lichen planus cum pigmentatione, pigmentsyphilis, etc.)

II. Hereditary
1. Nevus pigmentosus
2. Nevus spilus
3. Nevus of Ota
4. Ephelid

III. Skin Tumors
1. Melanoma
2. Basal cell carcinoma/epithelioma
3. Spitz nevus
4. Solar keratosis
5. Bowen's disease
6. Blue nevus
7. Others

luteal phases. Significant differences were only present in the increased levels of progesterone (P4) and 17OH progesterone in the plasma in the luteal phases of melasma patients as compared to the age-matched female controls without melasma (Fig. 1). Other hormones, such as estradiol, follicle stimulating hormone, luteinizing hormone, prolactin, androstendione, and cortisol (Fig. 2), showed no differences between groups during the ovarial and luteal phases. The increase in plasma progesterone may be attributed to the fact that melasma is exacerbated by pregnancy where plasma progesterone is increased or by contraceptive pills that occasionally contained progesterone; there is gradual decline of melasma after climacterium by 70 years of age.

Histopathology of melasma shows an increase in melanin pigments in the epidermal cells especially in the supranuclear region of the basal cells (Fig. 3). The number of epidermal melanocytes has not increased and, therefore, the hyperpigmentation of melasma is considered to be functional and reversible. Two links, however, are still missing: the connection to the increase in serum progesterone

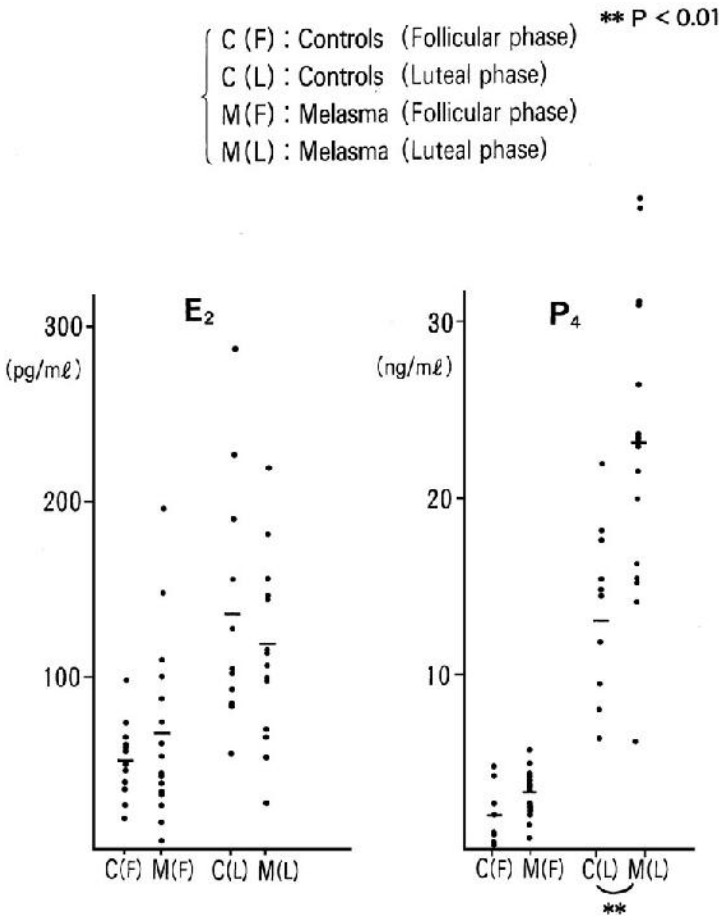


Figure 1 Serum progesterone (P4) and estradiol (E2) levels of melasma patients and matched controls in follicular and luteal phases.

since the intracellular receptor in the melanocytes is not known, and the photosensitivity of melasma patients has not been clarified.

When minimum erythema dosis (MED) was measured in melasma patients, 18 (24.7%) of the 73 melasma patients showed clear photohypersensitivity by lowered MED and minimum pigmentation dosis (MPD) to UVA and UVB. Further study showed that reactivity to UVA was normal but hypersensitivity to UVB was remarkable in all 15 patients. With such photohypersensitive melasma patients, MED was lowered to approximately one-third of normal persons in sum-

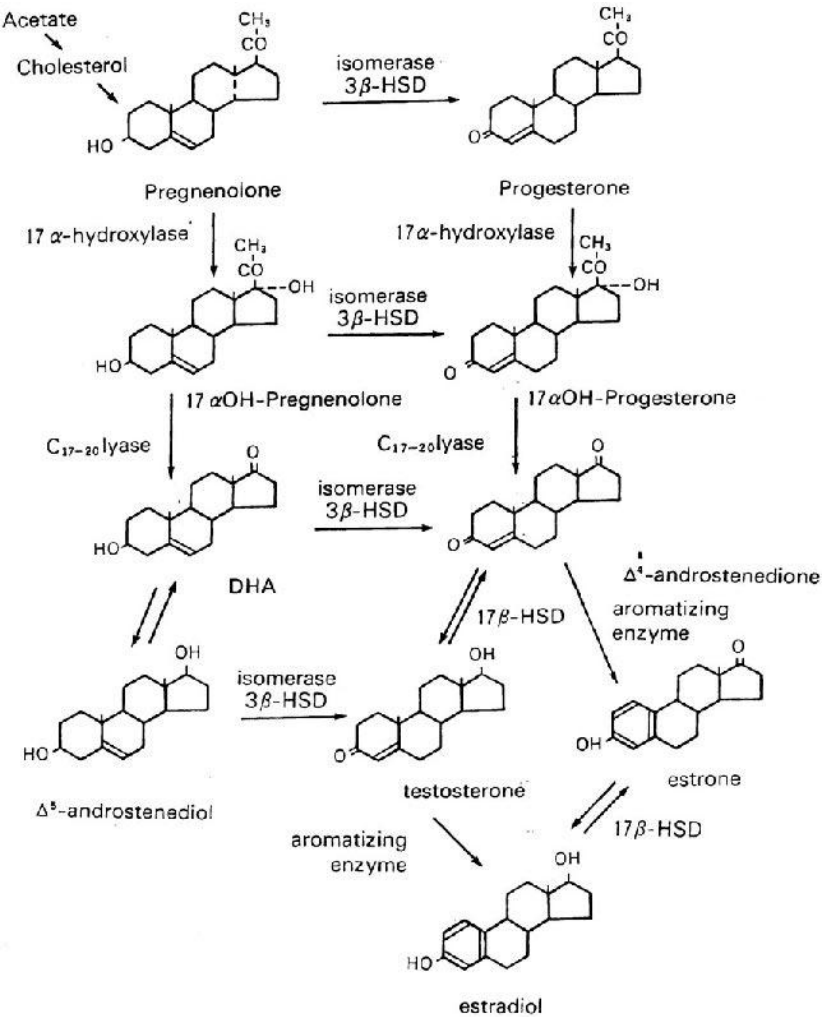


Figure 2 Biosynthesis of steroid hormones.

mer, and a palpable erythema was observed above 2 MEDs of UV-B which produced long-lasting hyperpigmentation for weeks. Therefore, 2 MEDs were almost equal to 1 minimum quaddel dosis (MQD) and to 1 MPD (Table 2; Fig. 4). All these patients did not have any medication when MED was measured, uro- and copro porphyrin levels were normal in urine, and the effect of common photoallergens such as musk ambrette or thiazides was denied. Plasma 17OH progester-

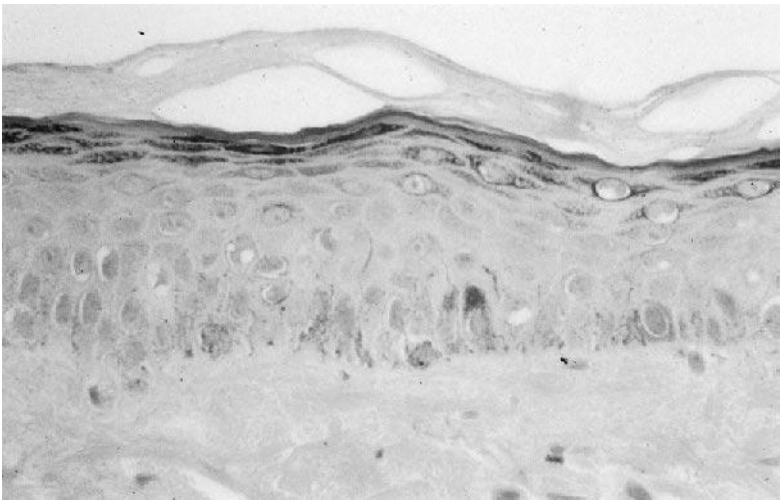


Figure 3 Histopathology of melasma shows increased melanin pigment at the basal layer and the lower part of prickle layer of the epidermis.

one levels were elevated only in one case, but nine other cases showed normal results when these photohypersensitive cases were again examined. Therefore, the mechanism of UVB photohypersensitivity in melasma should be investigated in the future.

Melasma has been regarded as an excellent target for newly developed depigmentation agents because many middle-aged melasma patients want to return their skin color to normal. Long-term therapy is necessary so that depigmentation occurs slowly, without provoking severe depigmentation (as with hydroquinone monobenzyl ether) or severe hyperpigmentation of ochronosis (as with hydroquinone at 2 ~ 4% concentrations under a tropical climate) (3). Historically, both disorders had been reported (4) and, therefore, both are disastrous pitfalls for those developing depigmentation agents.

First, unlike hydroquinone monobenzyl ether, the depigmentation agents under development should not kill melanocytes. Second, hydroquinone itself is not cytotoxic to melanocytes; however, it degenerates dermal elastic fibers under strong sunlight at high concentrations of 2–4%, which results in another disastrous strong brown hyperpigmentation called ochronosis (5). Therefore, the best depigmentation agent inhibit tyrosinase in melanocytes, and toxicity to epidermal cells, melanocytes, dermis, and other systemic organs is negligible. Also, depigmentation agents should not be strong sensitizers, oncogenic, or teratogenic. They should be stable chemically at least for more than a year.

Table 2 MED and MPD with Melasma Patients (1983 ~ 1987)

Apparatus: NS-9[a]
Results: 1 MPD ≒ 1 MQD ≒ 2 MED (general rule)

1. MED

	Shortened	Normal	Total
Spring	9	13	22
Summer	18	55	73
Autumn	0	3	3
Winter	1	10	11

2. MPD

	Shortened	Normal	Total
Spring	10	12	22
Summer	23	49	72
Autumn	0	3	3
Winter	4	7	11

[a] Light sources: { FL-20 BA-37, 20W × 2 (UV-A); FL-20 E , 20W × 2 (UV-B) }
Tube-skin distance: 10 cm
Automatic irradiation time: 10–90 s, at 10-s intervals
Performance
1. Normal individuals
{ MED: 70 ~ 90 s (Spring–Summer); MQD, MPD: more than 90 s. }
2. Photodermatitis patients
{ MED: shortened to 10–60 s; MQD, MPD: delayed erythema, etc., is detectable. }
NS-9 is a modified version of the previous type NCA-6, added with an inverter to shorten the irradiation time for MED.

Hydroquinone cream changes color from white to brown after 3–4 months; therefore, it can be produced at pharmacies and hospitals on the condition that it is disposed of after the color changes. Therefore, it cannot be used in cosmetics or cosmeceuticals. Hydroquinone cream is an excellent preparation for the treatment of melasma with or without mild chemical peeling (6,7). However, the color change and the production of ochronosis have inhibited its usage in cosmetics and cosmeceuticals.

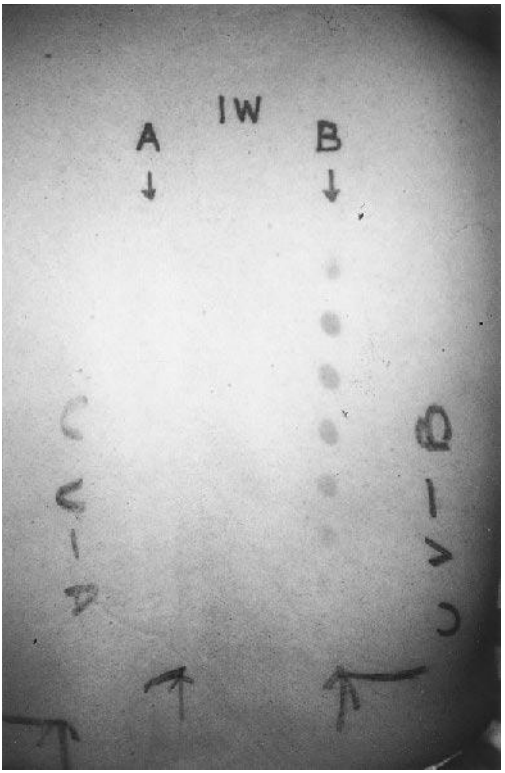


Figure 4 The results of MED and MPD (Table 2) showed that UVB hyperpigmentation was demonstrated at 1 week of UV irradiation. Note that no reaction occurred under UVA irradiation, even though the doses were the same.

SCREENING TESTS FOR DEPIGMENTATION AGENTS

A standard method for screening depigmentation agents is the isolated tyrosinase inhibition test. Mushroom tyrosinase has been commonly used, and the suppression of tyrosinase could be demonstrated when dose-dependent inhibition was demonstrated with hydroquinone as an effective control. Another kind of tyrosinase assay is noninhibitory or nonsuppressive-type reactions of melanogenesis. According to Mishima (8), melanogenesis can also be hindered by tyrosinase production inhibition, inhibition of tyrosinase transfer, and cytotoxic inhibition (Table 3). Cultured B-16 melanoma cells have been used in this field and are useful in demonstrating several new mechanisms of melanogenesis inhibition: glycosylation turned out to be another process of the production, along with matu-

Table 3 Mechanism of Melanogenesis Inhibition

Mechanisms	Example
1. Suppression of tyrosinase	Kojic acid
	Hydroquinone
	Ascorbic acid
	Arbutin
	Ellagic acid
2. Other mechanisms	
a. Decrease in tyrosinase synthesis	Biomein®
b. Decrease in tyrosinase transfer	Glucosamine
	Tunicamycin
c. Cytotoxicity to melanocytes	Hydroquinone monobenzylether
	APTA[a]

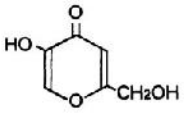
[a] n-2,4-Acetoxyphenyl thioethyl acetamide.

ration of melanogenesis. Its inhibition also decreased the amount of melanin, and depigmentation agents were also found. Tyrosinase activities in ribosomes and the production of premelanosomes can also be targets for melanin production inhibition (8). There are two melanins, eumelanin (black ~ brown) and pheomelanin (yellow or red), and eumelanin production inhibition is usually considered with depigmentation agents.

Dose-dependent reactions are requested for depigmenting agents in in vitro tests, like tyrosinase inhibition or B-16 melanoma cell assay. This is needed because melanogenesis inhibition increases in parallel with the concentration of the depigmentation agents in the medium. When some chemical is added to the medium and the inhibition of melanogenesis disappears, it means that the added substance (Fig. 5) could successfully block the active site of metabolism, and thus the mechanism of this depigmentation agent becomes quite clear.

An example is shown in Figure 6, where we can see that a dose-dependent melanogenesis inhibition of kojic acid was completely blocked when cupric acetate was added to the medium. These results showed that the main mechanism of kojic acid was to chelate copper ions that are indispensable for tyrosinase so that a remarkable decrease of its activity was seen by the addition of cupric acetate.

Streptomyces fervens produces melanin when it is cultured in a liquid medium, and the melanin synthesis can be inhibited by the presence of depigmentation agents. An example that also shows the dose-dependent effect of kojic acid can be seen in Figure 7. The important fact is that streptomyces was alive in all the culture medium, even though black eumelanin was not produced or decreased in production after kojic acid was added in various concentrations: when streptomyces was transferred to another culture medium without kojic acid, it produced



Molecular weight : 142.11

5-Hydroxy-2-(hydroxymethyl)-4-Pyrone

Procedure

glucose

← *Aspergillus albus*

crude KA — 30~32℃, aerobic conditions

↓ — Culture for 5 or 6 days

filtration

↓

charcoal treatment

↓

concentration

↓

recrystallization by water

↓

pure Kojic acid

only one peak

with gas chromatography

Figure 5 Kojic acid (KA).

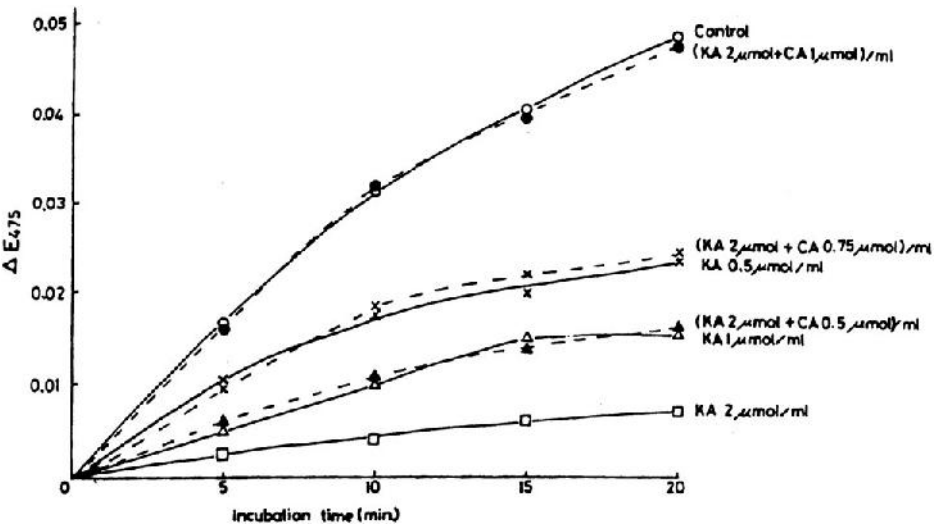


Figure 6 Suppression of melanogenesis. Reduction of tyrosinase (gold fish) inhibitory effect of kojic acid after pretreatment with cupric acetate. KA: Kojic acid; CA: cupric acid.

Figure 7 Streptomyces fervens produces melanin, and its melanin synthesis was inhibited by kojic acid at dose response, when the concentration of kojic acid increased from left to right. The left tube shows the color without kojic acid. Streptomyces was alive, even though melanin synthesis was inhibited.

Table 4 In Vitro and Animal Assays for Depigmentation Agents

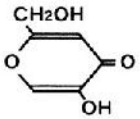
Assays with which melanogenesis inhibition was confirmed	Depigmentation agents
1. Tyrosinase inhibition test 2. Melanin reduction of B-16 melanoma cells	Kojic acid Hydroquinone Arbutin Ellagic acid 4n-butylresorcinol Ascorbic acid Liquiritin
3. Reduction of melanin pigments of *Streptomyces ferbens*	Kojic acid Hydroquinone
4. Reduction of melanin pigments of black goldfish	Ascorbic acid Kojic acid (Fig. 3)
5. Reduction of melanin pigments of pigmented mammals (C57 black mouse, Yucatan pig, etc.)	APTA[a] (topical application or intraperitoneal injection)

[a] n-2,4-Acetoxyphenyl thioethyl acetamide.

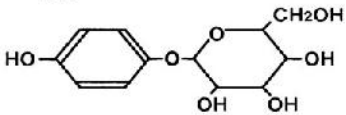
Table 5 Chemical Structures of Main Depigmentation Agents

1. Hydroquinone

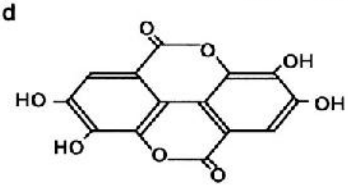
2. Kojic acid



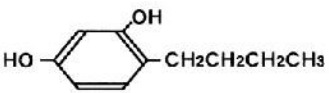
3. Arbutin



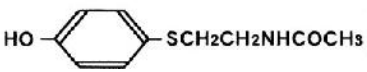
4. Ellagic acid



5. Rucinol
(4-n-Butylresorcinol)



6. N-Ac-4-S CAP
(N-2,4-acetoxyphenyl thioethyl acetamid)



melanin, turning the color of the medium to black again. Various assays to detect depigmentation agents (9–12) are listed in Table 4, and the chemical structures of main depigmentation agents are shown in Table 5.

Cultured B-16 melanoma cells are also excellent material for visually confirming the melanogenesis inhibition in vitro. A recommended method is to culture B-16 cells in Eagle's MEM with 10% fetal bovine serum, and depigmentation agents are added in the culture medium at different concentrations. After 5 days of the culture, the cells are fixed by formalin and stained by ammonical silver nitrate, then premelanosome can be visually stained in black. When the

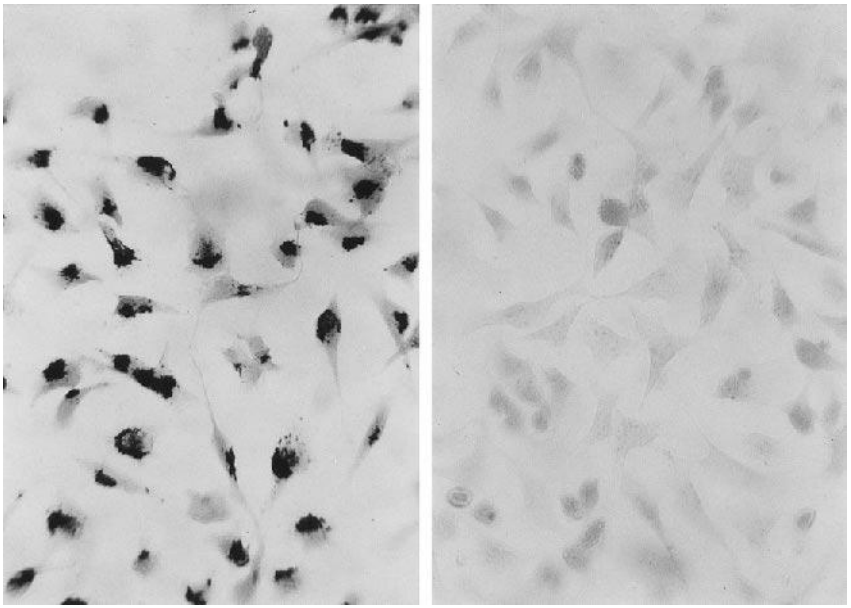


Figure 8 Assay of melanogenesis inhibition using B-16 melanoma cells. Right side shows inhibition effect of kojic acid put into the culture medium at 2.5 mM concentration. Premelanosome reaction is negative. Left side shows control without kojic acid, and premelanosome is clearly seen.

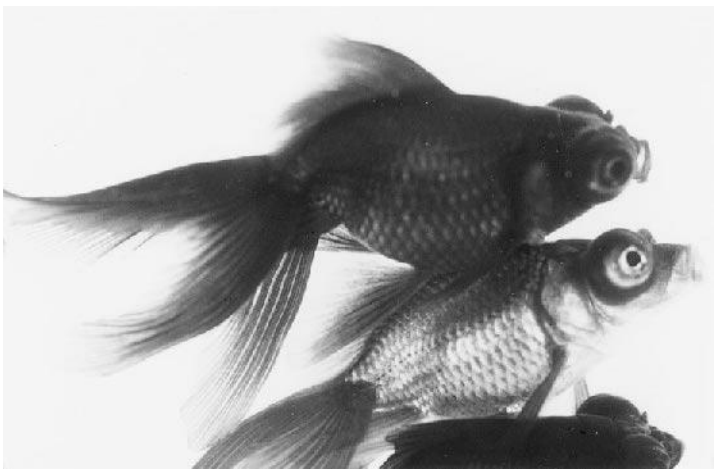


Figure 9 Black gold fish (upper and bottom as controls) changed color form black to brown, when kojic acid was added in the water at 0.25% for a month (middle fish).

cells are alive, and such premelanosome stain is negative with the presence of depigmentation agents, melanogenesis is recognized as having been successfully inhibited (Fig. 8).

More dramatic effects of melanogenesis inhibition can be recognized when a depigmentation agent is added to the water in which black goldfish are kept. The addition of kojic acid required a month or two for the black goldfish to turn to yellowish brown; since they were alive and vivid, this demonstrated that only melanogenesis was inhibited, not systemic metabolism (Fig. 9).

CLINICAL EVALUATION

Depigmentation agents can be screened in vivo by tyrosinase inhibition tests or various other methods that clearly demonstrate the inhibition of melanogenesis; however, what is most important is that not only they show definite melanogenesis inhibition in vitro, but also they improve the hyperpigmentation of melasma in clinical evaluation. When there is no clinical effect of depigmentation, they are of course useless, even though they showed excellent results in vivo trials. Laser is not effective to melasma, and is very effective to solar lentigo and to nevus of Ota to which depigmentation agents are less effective or ineffective. Therefore, the best target for depigmentation agents is apparently melasma.

First, for that purpose, depigmentation agents should be mixed in vehicles, normally creams or lotions, without any alteration of the color or the effectiveness. They should be put into production without producing impurities. They should pass acute, subacute, and chronic toxicity tests, skin and eye irritation tests, skin sensitization tests (maximization and similar tests), oncogenicity tests (Ames test, micronuclei tests, carcinogenicity tests), teratogenicity tests, and stability tests. These tests are all required to develop new drugs and likewise with depigmentation agents. It is because depigmentation agents require several months to exhibit their effects and consumers may use them for several months or even several years.

Double-blind clinical tests for melasma usually are not appropriate because as it takes more than 3 months for the effect to be recognized. Actually, depigmentation agents like kojic acid, hydroquinone, and arbutin can improve the brown hyperpigmentation of melasma by continual usage for 3–12 months. Theoretically it is possible to give active depigmentation agents to one group while a second group is given a placebo cream for 3 to 12 months (13,14); there should be no significant differences between the backgrounds of the melasma patients as to age, severity, and sun exposure. It is ethically acceptable to use a placebo when another, effective treatment is given. However, when melasma patients are involved in the clinical trial, they have the right to see improvement in a short period of time. Therefore, the long-term use of placebo cream was abandoned

because it apparently deceived patients who anticipated the effect. Double-blind tests are alright when the test ends in a week or so (as with corticosteroid ointments or antibiotics), especially when some another reliable basic treatment is given or the placebo is a competing drug having a definite effect.

Hydroquinone cream is not suitable as an active placebo because the brown color change after a few months indicate that it is hydroquinone: this is an open test (6), not a blind test.

With cosmeceuticals, double-blind tests have not always been demanded, presumably because they were not as strong as drugs and had mild effects not detectable in a short period. When some medical effects are exhibited after long-time usage, double-blind tests are difficult and, in some instances, not ethical when the patients are to be given a placebo with no effect for months. Therefore, double-blind tests should be introduced with care with cosmeceuticals with mild and slow effects.

The evaluation of the treatment of pigmentary disorders of the face is not easy. With melasma, the brown pigmentation fades so slowly that patients often do not recognize the effects of depigmentation agents after 6 months of continual, twice-a-day application. The best way to evaluate is to take color photographs of the faces of melasma patients from three angles—front, 45° right, and 45° left. When the same camera, flashlight, and color film are used, the effect of depigmentation agents can surely be recognized (7,13,14). First the color of the melasma turns from brown to yellowish brown or normal skin color, and second, the contrast at the border of the melasma becomes obscure. When colorimetry is used, it is possible to recognize the change of tint, but when the place of measurement differs at times of measurement, correct change of color is difficult to be obtained. Mapping the human cheeks and forehead to determine the same spots at each time of measurement is usually difficult.

On the other hand, pattern recognition using color photographs from the same three angles of the face is much easier (13,14). When past color photographs from the same three angles of the patient's face are shown when the patient comes for evaluation, the effect of whitening is easily recognized. At the very least, classification (''cured, almost cured, remarkably effective, effective, slightly effective, no effect, and exacerbation'') is possible. Tables 6 and 7 and Figures 10 and 11 illustrate such evaluations.

Similar evaluation is possible with solar lentigo, ephelid, and pgimented cosmetic dermatitis; however, at the beginning of 21st century, the best treatment for solar lentigo is laser. Solar lentigo is due to the local proliferation of melanocytes; therefore, the destruciton of melanocytes without giving serious damage to epidermal cells is ideal. Fortunately, lasers can do this, and iatrogenic vitiligo is not formed by this treatment.

Pigmented cosmetic dermatitis is sometimes similar to melasma, when reticular hyperpigmentation is lacking and moderate diffuse brown hyperpigmenta-

Table 6 Effect of 1% Kojic Acid Cream I on Melasma Patients (1982)

Effect	Cases treated	Duration of treatment (months, mean ± SD)	%
Complete cure	0	—	0.0
Remarkably improved	37	13.9 ± 4.3	95.5
Improved	26	9.5 ± 5.5	
No effect	0	—	0.0
Worsened	3	5.3 ± 4.9	4.5
Total	66	11.8 ± 5.4	100.0

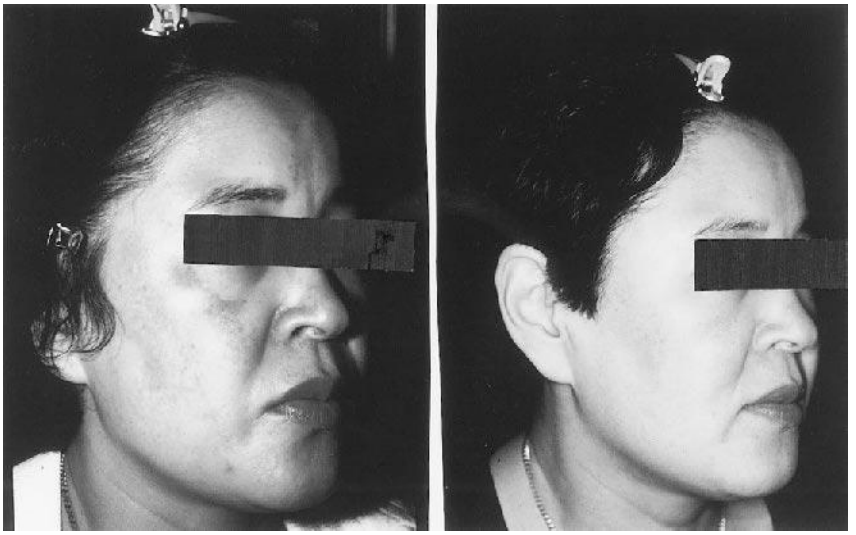
Source: Ref. 13.

tion is the main symptom. Biopsy shows basal liquefaction of the epidermis along with incontinentia pigmenti histologica and small amount of cellular infiltration composed of lymphocytes and histiocytes in the upper dermis, not like the basal hyperpigmentation of the basal layer cells of melasma. The most important and essential treatment for pigmented cosmetic dermatitis is not the use of depigmentation agents, but of patch testing of cosmetic series allergens including phenylazo-naphthol, D&C Red 31, D&C Yellow No. 11, benzyl salicylate, jasmin absolute, ylangylang oil, geraniol, sandalwood oil, artificial sandalwood, cinnamic alcohol, fragrance mix 1 and 2, etc. (15). The reading should be performed on the second, third, and seventh day so as not to overlook slow, but strong, allergic reactions. The exclusive use of allergen-free soaps and cosmetics for a year or

Table 7 Effect of 1% Kojic Acid Cream II with an Improved Base Cream on Melasma Patients (1994)

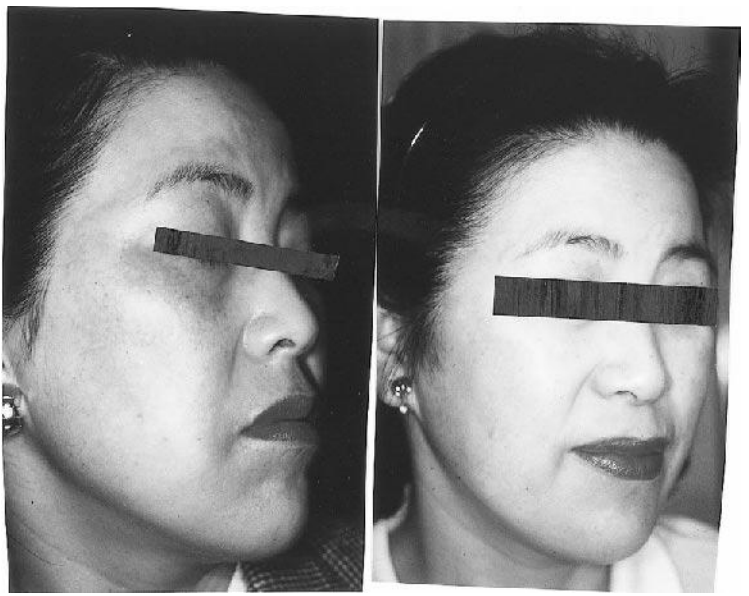
Effect	Cases treated	Duration of treatment (months, mean)	%
Complete cure	0	—	0.0
Remarkably improved	48	11.5	80.9
Improved	58	11.1	
No effect	25	12.1	19.1
Worsened	0	—	0.0
Total	131	11.4	100.0

Side effect: Those who were contact sensitized by having previously used kojic acid cream containing betacyclodextrin also developed erythema and itching by the usage of 1% kojic acid cream II. The rate of the dermatitis was 2 out of 131 patients in the table (1.5%). Those who had not used betacyclodextrin-containing kojic acid cream had not produced contact dermatitis.



(a) (b)

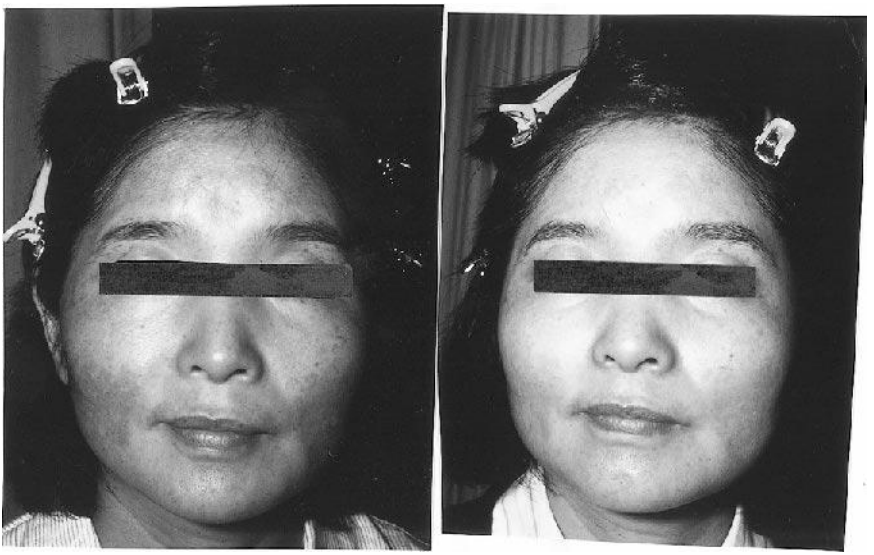
Figure 10 Hyperpigmentation in a 42-year-old melasma patient (a) decreased remarkably by the application of 1% kojic acid cream twice a day for a year (b).



(a) (b)

Figure 11 Hyperpigmentation in a 48-year-old melasma patient (a) decreased remarkably by the application of 1% kojic acid cream twice a day for one-and-a-half years (b).

more restores the normal skin color of the patient (15,16). It is impossible to treat the disease with corticosteroid ointments, even though it is a kind of allergic contact dermatitis, because only a small amount of cosmetic allergens invade the skin everyday and these are enough to provoke the disease and maintain the hyperpigmentation. With this disease, allergen control (15) is the treatment of choice; however, the additional use of a depigmentation agent accerelates the cure, presumably because the long-term inflammation at the basal layer of the epidermis enhances the melanin production and increases brown hyperpigmentation. An important fact is that sometimes melasma is complicated by pigmented cosmetic dermatitis (which is shown by biopsy with the presence of incontinentia pigmenti histologica and the inflammatory infiltrates in the upper dermis, by occasional slight erythema with itching on the face, and by positive patch test results



(a) (b)

Figure 12 A 44-year-old female who suffered from melasma of the face complicated by pigmented cosmetic dermatitis (a). In addition to noninflammatory diffuse brown hyperpigmentation, she occasionally produced irregular brown hyperpigmentation of the face. Trepan biopsy showed not only basal hyperpigmentation of the epidermis, but also cellular infiltration composed of lymphocytes and histiocytes in the upper dermis. Patch test revealed that she was strongly sensitized to d-hydroxycitronellal. Allergen control by the exclusive use of allergen-free cosmetics and soaps to avoid contact with d-hydroxycitronellal could remarkably cure the inflammatory hyperpigmentation, and the remaining diffuse pigmentation of melasma was almost cured by the usage of 1% kojic acid cream for 10 months (b).

of common cosmetic allergens). It is understandable that when melasma patients try to conceal the pigmentation by frequent use of various cosmetics, some of them become sensitized to cosmetic components, which results in the complication of pigmented cosmetic dermatitis. Such a case is shown in Figure 12.

THE CASE OF KOJIC ACID

In 1977, a project was started to find out the cause of melasma and its reliable treatment. From 1970 to 1974, most of the causative contact cosmetic allergens that produce pigmented cosmetic dermatitis had been discovered; by 1977, the disease, which had been incurable prior to 1971, was cured, not by medication but by the exclusive use of allergen-free cosmetics and soaps. This usage of allergen-free cosmetics and soaps was designated as the allergen control system (ACS) (15). The effect of ACS had been so dramatic that a number of melasma patients whose outlook was somewhat similar to pigmented cosmetic dermatitis visited Saiseikai Central Hospital in Tokyo everyday, where ACS was invented and reported on by the mass media.

The introduction of system engineering to develop a subsystem to find the causes and how to eliminate them was key to solving the problem of pigmented cosmetic dermatitis. A new team was formed to solve the problem of melasma adopting a similar system engineering prototype; a team investigated the role of female hormones analyzing the plasma of the both melasma patients and age-matched melasma-free women, at the seventh days of both the ovarial and luteal phases (1). The second team investigated photohypersensitivity by an automatic UVA and B irradiator with melasma patients. The third group started to develop a cream containing a melanogenesis inhibitor, a depigmentation agent. A plan to develop 1% hydroquinone cream was rejected by the Ministry of Health and Welfare (MHW) of Japan because, at that time, the erroneous idea that the serious and persistent leukomelanoderma caused by a depigmentation agent (hydroquinone monobenzyl ether cream banned in 1957) was an effect of hydroquinone released from hydroquinone monobenzylether. Therefore, among the known chemicals that were tyrosinase inhibitors, kojic acid was selected as the new depigmentation agent, because of its extremely long history of safe ingestion. In Japanese, kojic means ferment and had been used to brew Japanese liquor (Sake) made from rice. Pure kojic acid could be produced from glucose by fermentation and various assays to determine the mechanisms of depigmentation along with the necessary safety evaluation tests were performed. The results are shown in Figure 13 and Table 8, showing its confirmed mechanism of action and its safety.

The initial clinical evaluation of kojic acid cream showed that 1% cream was better than 2.3% (saturated) cream, because with the latter, crystallized kojic

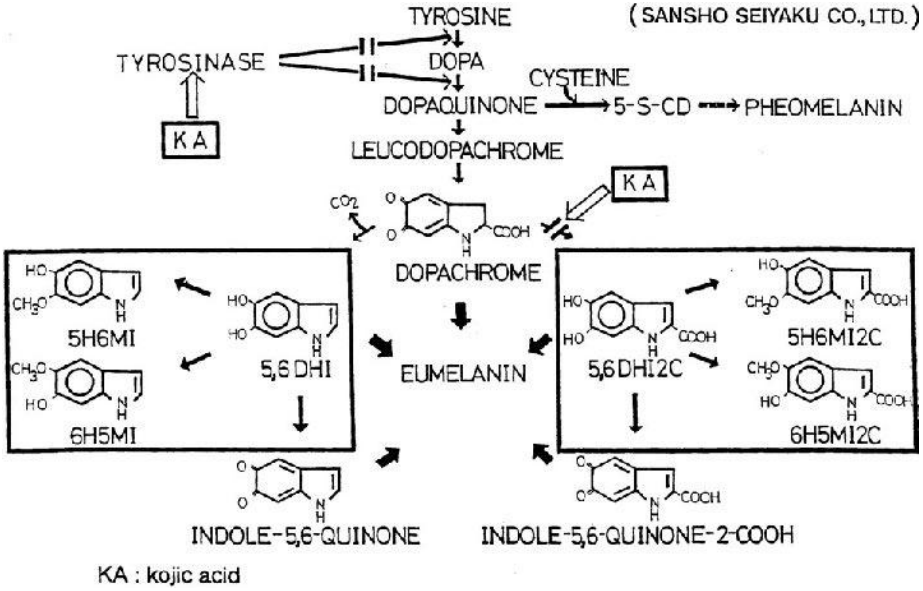


Figure 13 Inhibitory action of kojic acid on melanin polymer formation.

acid gradually appeared and the effect of the improvement was inferior to 1% cream, with which kojic acid melted very well to the vehicle. At this time, the first and the second trials had almost ended, having shown that the cause of melasma was most likely the increase in plasma P4 levels at the luteal phase, and also that 20% of melasma patients were strongly hypersensitive to UVB rays.

Sun protection was introduced to those patients who showed such photohypersensitivity. Some melasma patients were remarkably improved by the continual daily application of 1% kojic acid cream for 6 months, however, after, day of exposure to sunlight (through such activities as golf, fishing, mountaineering, etc.), the melasma reappeared.

The effect of whitening was steady but too slow with this initial 1% preparation of kojic acid. ^{14}C-labeled kojic acid cream was observed to be quickly absorbed from the skin to the liver, intestines, and kidneys in mice. When the absorption was thus quick, the depigmentation agent did not stay at the epidermis where it had its target organ, melanocytes, for a long enough time to inhibit melanogenesis. Therefore, the second preparation conceived was 1% kojic acid cream wherein kojic acid was mixed with betacyclodextrin to slow absorption into the dermis. This successfully sped up the whitening effect; however, contact

Table 8 Toxicity of Kojic Acid (KA)

1. LD_{50}

	Mice	Rats
Subcutaneous	2050~2080 mg/kg	3010~3080 mg/kg
Oral	2650~2920 mg/kg	2260~3040 mg/kg

2. Chronic toxicity (rats)

Oral, 125, 250, 500, 1000 mg/kg for 26 weeks $\frac{\text{death}}{18}\ \frac{0}{}$

3. Teratogenicity — Mice: None; Rabbits: None

4. Mutagenicity

Ames test	(−) up to 1000 μg/plate
Micronuclei test	Negative
Dominant lethal test (mice)	Negative

5. Skin irritation test

Draize method (rabbits) — Negative $\left(\frac{0}{6}\right)$
50% KA aq., Patch test for 24 h.

Chronic irritation test (rabbits) — Negative $\left(\frac{0}{9}\right)$
Patch test for 6 h ×30 days

Phototoxicity test (guinea pigs): (−)
5.0% KA ethanol + UVA × 5 days

6. Maximization test (guinea pigs): $\frac{0}{10}$

7. Human skin closed patch test for 48 h

3% KA aq.	$\frac{1}{30}$	?(+),	$\frac{29}{30}$	Negative
1% KA aq.	$\frac{2}{30}$	?(+),	$\frac{28}{30}$	Negative

sensitization to kojic acid occurred (17). Betacyclodextrin turned out to be a new adjuvant and, consequently, it was removed; the base cream was improved to delay the absorption without using betacyclodextrin. Contact hypersensitivity to kojic acid is rare today. Effects as in Table 7 have been followed up every year, and 30 cases of melasma who had used 1% kojic acid cream for more than 2 years were examined (CBC, liver function tests, and other systemic abnormalities including carcinogenesis). No abnormal results were demonstrated, except in one person with meningioma, which was considered as coincidental. Such follow-up is always necessary whenever a new drug or cosmeceutical is introduced. Today,

new depigmentation agents, kojic acid, arbutin, and rucinol, are commercially distributed as cosmeceuticals (the Japanese term is quasidrug). Several others may be introduced in the future (18,19).

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