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Letter

Depigmentation of the skin induced by 4-(4-hydroxyphenyl)-2-butanol is spontaneously re-pigmented in brown and black guinea pigs

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ABSTRACT — Chemically induced depigmentation of the skin, which occurs following exposure (application or inhalation) to a depigmenting agent, is a disease with clinical findings similar to vitiligo. Recently, skin depigmentation possibly resulting from exposure to 4-(4-hydroxyphenyl)-2-butanol (HPB) was reported in humans. However, the role of HPB as the causative material of this skin depigmentation was not clear. To evaluate whether HPB has the potential for skin depigmentation, we characterized its effects on the skin of pigmented guinea pigs. Following exposure to 30% HPB 3 times/day for about 20 days, we found that obvious skin depigmentation was induced in brown and black guinea pigs. In the depigmented skin, there was a marked reduction in melanin pigment, and decreased numbers of DOPA and S-100 positive epidermal melanocytes were observed histologically. In addition, the depigmentation gradually recovered spontaneously and the number of melanocytes in the skin also increased after terminating the application of HPB. Complete re-pigmentation needed 31 to 70 days to return to the original baseline level. These data indicate that skin depigmentation is induced by the toxicity of HPB to epidermal melanocytes, and that the induced skin depigmentation can recover by terminating the application of HPB.

Key words: Depigmentation, 4-(4-hydroxyphenyl)-2-butanol, Melanin, Melanocyte, Guinea pigs, Skin

INTRODUCTION

Depigmentation of the skin has been reported to be induced by damage to melanocytes in exposed sites, which then cannot produce melanin pigment following chemical exposure of the skin. For example, skin depigmentation due to exposure to a skin-bleaching cream containing hydroquinone (HQ) (Arndt and Fitzpatrick, 1965), occupational depigmentation of the hands caused by a HQ-containing photographic developer (Frenk and Loi-Zedda, 1980; Kersey and Stevenson, 1981), occupational depigmentation caused by an o-phenylphenol-containing microbiocide in a hospital (Kahn, 1970) and depigmentation of the hands and forearms caused by 4-*tert*-butylphenol in a factory manufacturing resin (Ebner *et al.*, 1979; Gebhart *et al.*, 1980) have been reported. Skin depigmentation was also induced in workers engaged in the manufacturing process of raspberry ketone (RK, 4-(4-hydroxyphenyl)-2-butanone) (Fukuda *et al.*, 1998b). In addition to phenols and catechols, other chemicals, such as sulfhydryls and p-phenylenediamine, have also been reported to cause skin depigmentation (Boissy and Manga, 2004).

Regarding evaluation methods for chemically induced depigmentation, some assays using pigmented animals have been reported. To assess chemically induced depigmentation, pigmented guinea pigs are ideal because the localization of epidermal melanocytes in guinea pig skin is similar to that of humans. To assess HQ and phenylhydroquinone (PHQ) depigmentation, continuous treatment models using guinea pigs with black skin were reported (Bleehen *et al.*, 1968; Jimbow *et al.*, 1974; Tayama and Takahama, 2002). In those models, visual grading of the skin, the number of dopa-positive epidermal melanocytes and histological analysis were evaluated. In addition, the use of pigmented mice to examine the depigmenta-

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tion caused by RK or monobenzyl ether of hydroquinone (MBEH) was also reported (Fukuda *et al.*, 1998a; Zhu *et al.*, 2013). However, since melanocytes are not distributed in the epidermis of normal mice except for the ears and tail, the endpoints of depigmentation were bleaching of the ears and tail (Zhu *et al.*, 2013) or the melanin content in the hair (Fukuda *et al.*, 1998a).

4-(4-hydroxyphenyl)-2-butanol (HPB) has been formulated in topical products used by subjects concerned about pigmented spots on their skin (e.g., chloasma and ephelides). A recent report (Nishigori *et al.*, 2014) suggested an association of HPB with skin depigmentation. In this study, we evaluated whether HPB has the potential to depigment skin. To assess the depigmentation potential of HPB, we chose the pigmented guinea pig model described above. However, it was difficult to obtain black guinea pigs and thus, we also examined brown guinea pigs, which have a brighter skin color and fewer epidermal melanocytes than black guinea pigs.

In previous studies, depigmentation was reported to occur in about one month. In our study, we also evaluated the time for depigmentation when HPB was applied 3 times a day, which is an excessive experimental condition that results in a 15-fold higher exposure concentration than estimated use conditions.

MATERIALS AND METHODS

Chemicals

HPB was prepared by reducing RK with Raney Ni in EtOH (Carruthers, 1978). The purity was 100%, and the chemical structure is shown in Fig. 1. Ethanol (EtOH) as the vehicle was purchased from Wako Pure Chemical (Osaka, Japan).

Animals

Five female brown guinea pigs (kwl:A-1 strain, 7-weeks old), with brown hair and skin, were purchased from Tokyo Laboratory Animals Science (Tokyo, Japan). One black guinea pig (JY-4 strain, 1.5 years-old), with black hair and gray-black skin, was obtained from the Tokyo Metropolitan Institute of Public Health (Tokyo, Japan). All animals were housed with free access to standard food pellets and water. During the experiments, the animals were cared for in the experimental animal facility of the Kao Corporation. The Animal Care Committee of the Kao Corporation approved this study, and all experiments strictly followed the guidelines of that Committee.

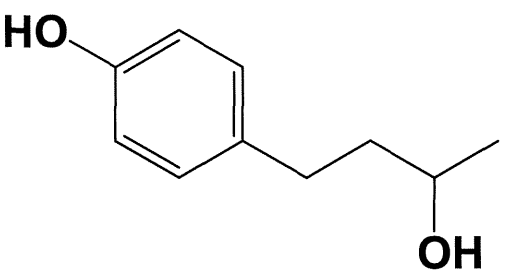

Fig. 1. Chemical structure of 4-(4-hydroxyphenyl)-2-butanol (HPB).

Experimental design

The dorsal hairs of both strains of guinea pigs were cut with electric clippers and were shaved daily. Six dorsal areas (2 x 2 cm per area) on the back of each animal were used, as shown schematically in Fig. 2. All HPB solutions to be tested were prepared in 50% EtOH (ethanol:water = 1:1) daily. Twenty microliter aliquots of each test solution were applied 3 times per day to the appropriate area on the back of each animal.

In the brown guinea pigs, we created 6 treatment areas. Two treatment areas were exposed to 0.75 J/cm^2 UVB irradiation with an FL20SE lamp (Toshiba, Tokyo, Japan, wavelength spectrum 275-380 nm, peak 315 nm) 5 days before beginning the experiment, and then those 2 areas were treated with 30% HPB. Two other areas were only UV-treated. The final 2 areas remained intact with no treatment of any kind. One area from each pair of 2 areas was biopsied at the end of the study, and the other area was used to evaluate re-pigmentation (Fig. 2). To induce sufficient depigmentation, HPB was applied for 30, 40, 50, 60 and 97 days. To observe re-pigmentation, the HPB applications were terminated on days 31, 41, 51, 61 and 98. The re-pigmentation areas were then observed until the pigmentation returned to the original baseline level.

As for the study with the black guinea pig, a 30% HPB solution was applied continuously for 21 days to 2 areas. To observe re-pigmentation, the HPB application was stopped at day 22. One of the pair of 2 areas was biopsied at day 22 and the other was observed until the pigmentation returned to the baseline level.

Skin color/visual grading

Skin erythema and depigmentation were graded each day as negligible (–), slight (±), moderate (+) or marked (++), according to a previous report (Tayama and Takahama, 2002). Briefly, skin color similar to the control areas was defined as negligible, otherwise it was defined as slight, moderate or marked in accordance with the difference in color relative to the control area.

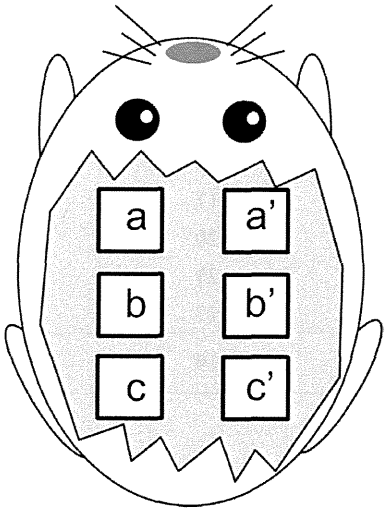

Fig. 2. Schematic representation for the fixed-dose experiment in brown guinea pigs. a: UV-treated and HPB-applied area (for biopsy), a': UV-treated and HPB-applied area (for recovery observation), b: no treatment area (for biopsy), b': no treatment area (for recovery observation), c: only UV-treated area (for biopsy), c': only UV-treated area (for recovery observation).

Colorimetric measurements

A tristimulus colorimeter (Chromameter, CR-300, Minolta, Tokyo, Japan) was used to evaluate brightness changes of the skin. Color is expressed using the L*a*b* system (Robertson, 1977). In this study, the L* value (lightness) was used, and changes in this parameter are used as an indicator of skin depigmentation (Seitz and Whitmore, 1988). The L* value was measured in each application area (automatic averaging 3 times per point). The mean value of the application area in each animal was obtained from more than 3 animals.

Histological analysis

Skin samples were taken using a dermapunch (5 mm diameter, Maruho, Osaka, Japan) from isoflurane (Forene, Abbott Japan, Tokyo, Japan)-anesthetized guinea pigs.

For split-dopa preparations, 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA, Wako Pure Chemicals) was dissolved in phosphate buffered saline and split-tissue samples were prepared according to the method described in Staricco and Pinkus (1957). The number of whole dopa-positive melanocytes in each sample was counted using a light microscope (Biophoto or Optiphot-2, Nikon, Tokyo, Japan) and cell numbers were calculated per square mm.

For paraffin-embedded sections, the skin samples were fixed overnight in neutral-buffered 10% formalin (Kokusan Chemical, Tokyo, Japan) and were then embedded in paraffin (Fisher Scientific, Pittsburgh, PA, USA). Paraffin-embedded sections of vertical skin samples were prepared in two ways: one involved histopathological examination using Hematoxylin and Eosin (HE, Muto Pure Chemical, Tokyo, Japan) and Fontana–Masson (FM) staining as a marker for melanin granules; and the other involved the immunohistochemical examination of S-100 protein (polyclonal antibody, Code No. z0311; Dako Co., Glostrup, Denmark) as a marker for melanocytes in the epidermis. The primary antibody for S-100 was diluted at a ratio of 1:2,400 and was reacted for 50 min at room temperature after the specimen was treated with 3% H_2O_2 for 90 min at 55°C. Sections from both groups were stained with FM and S-100, and were counterstained using Kernechtrot solution (Merck, Darmstadt, Germany) and hematoxylin, respectively.

Statistics

Significance of differences was calculated by Student's t-test (Microsoft Excel). A p-value of ≤ 0.01 is considered statistically significant.

RESULTS

Induction and recovery of depigmentation caused by HPB

To ascertain whether HPB has a depigmenting activity on melanocytes, a 30% solution of HPB was applied topically to brown and black guinea pigs continuously for up to a maximum of 97 days. A slight depigmentation (±) at the HPB-treated sites appeared on day 9 in 4 guinea pigs and was found in all 5 guinea pigs by day 10 (Table 1). With further treatment, the depigmentation gradually increased. The appearance of marked depigmentation (++) was observed between 17 to 21 days of treatment (Table 1). After the treatment of HPB was discontinued, the depigmentation disappeared over time. The re-pigmentation took 31 to 52 days from the day of HPB withdrawal to reach the same level as untreated skin (Table 1). Representative examples of baseline, depigmentation and re-pigmentation are shown in Fig. 3C through E. As for colorimetric measurements, the L* value (skin brightness) significantly increased at sites pre-treated with UV and then treated with HPB compared with the only UV-treated areas on days 14 and 21 (Fig. 3A). In addition, the L* values in HPB + UV-treated areas also increased compared to untreated skin on days 28 and 35. Continuous treatment with HPB sustained this augmentation (Fig. 3A). No skin erythema was induced in any of the animals (data not shown).

Table 1. Depigmentation by HPB application and repigmentation in guinea pigs

Guinea pig skin color	No.	HPB Concentration	Application time / total application number (3 times per day)	Day of first appearance of slight depigmentation (±)	Day of first appearance of marked depigmentation (++)	Day to complete repigmentation post PHB withdrawal
Brown (N = 5)	1	30%	30 days/90	9	20	52
	2	30%	40 days/120	9	17	35
	3	30%	50 days/150	9	20	41
	4	30%	60 days/180	10	21	31
	5	30%	97 days/291	9	17	44
Black (N = 1)	1	30%	21 days/63	5	19	70

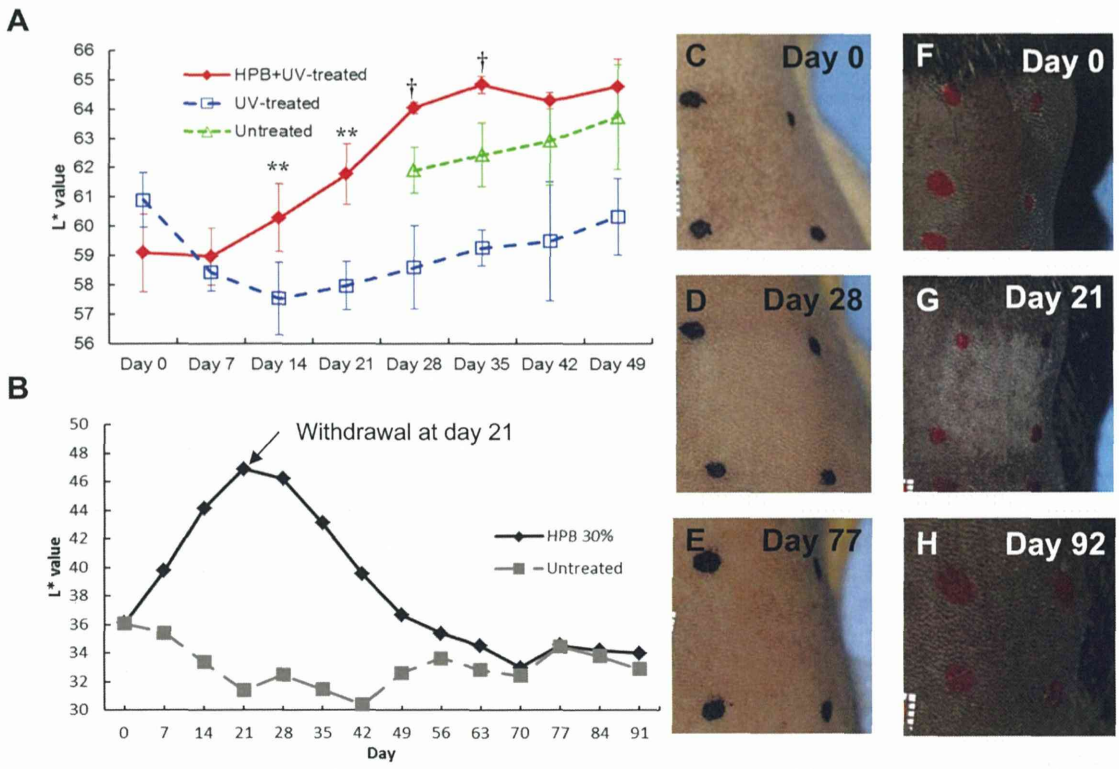

Fig. 3. Changes of skin brightness caused by HPB. (A) L*values of 3 dorsal areas in brown guinea pigs. Values are shown as means ± S.D. (Day 0-28, n = 5; Day 35, n = 4; Day 42-49, n = 3). (B) Time course of L*values for skin treated with 30% HPB and untreated skin of the black guinea pig. HPB was withdrawn on day 21. Representative photographs of dorsal skin from a brown (C-E) and a black (F-H) guinea pig. (C) UV-treated skin; (F) Untreated skin; (D, G) Depigmented skin; (E, H) Repigmented skin. ** $p < 0.01$ (HPB+UV-treated vs only UV-treated), † $p < 0.01$ (HPB+UV-treated vs untreated).

In the black guinea pig model, a slight depigmentation (±) of the sites treated with 30% HPB was first observed on day 5 and a marked depigmentation (++) was observed on day 19 (Table 1). It took 70 days for re-pigmentation to return to the same level as untreated skin after the treatment of HPB was discontinued (Table 1, Fig. 3F-H). As for the colorimetric measurements, the L*value increased up to day 21 post-withdrawal of HPB, after which this value decreased gradually to the baseline level (Table 1, Fig. 3B). Visual grading followed a pattern similar to the

Table 2. The number of dopa-positive melanocyte cells in split-epidermis

Guinea pig	Day	Depigmentation grade	Treatment	The number of dopa-positive melanocytes per mm^2
Brown (No. 2)	0	-	UV	99
	41	-	None	14
	41	-	UV	49
	41	++	UV+HPB	0.66
	78	-	UV +HPB (withdrawn on day 40)	31
Black	0	-	None	90
	22	++	HPB	2.2
	92	-	HPB (withdrawn on day 21)	24

L* value, which indicated that the application of HPB induced the skin depigmentation and that withdrawal of HPB resulted in re-pigmentation.

Quantification of dopa-positive melanocytes in the epidermis

Table 2 shows the representative number of dopa-positive melanocytes. In brown guinea pigs, the number of dopa-positive melanocytes per square mm was 99 in the only UV-treated site on day 0. The numbers of dopa-positive melanocytes decreased to 49, 14 and 0.66 in the only UV-treated, untreated and UV + HPB-treated depigmented skin on day 41, respectively. The number of dopa-positive melanocytes (0.66) in the UV + HPB-treated area was largely eliminated on day 41, although the number of dopa-positive melanocytes in the only UV-treated area was reduced by about 50%. Moreover, dopa-positive cells in the UV + HPB-treated area were clearly fewer than in the untreated site. The number of dopa-positive melanocytes per square mm was 31 in the UV + HPB-treated area on day 78 post-withdrawal of HPB and when the depigmentation had disappeared; which was more than the untreated site. Taken together, these results suggest that the number of dopa-positive melanocytes was markedly decreased and then increased along with the depigmentation caused by HPB and the subsequent re-pigmentation.

In the black guinea pig, the number of dopa-positive melanocytes per square mm was 90 on the day before the beginning of the experiment and decreased to 2.2 after treatment with HPB for 22 days (Table 2). However, the number of dopa-positive melanocytes increased to 24 after 69 days post-withdrawal of HPB. Thus, dopa-positive melanocytes were markedly decreased and then increased with the application or the removal of HPB, respectively, just as occurred in the brown guinea pigs.

Localization of melanocytes and melanin

The immunohistochemical localization of melanocytes and melanin content in brown guinea pigs and the black guinea pig (vertical sections) are shown in Figs. 4 and 5, respectively. The number of S-100 positive melanocytes in the basal layer and the quantity of melanin granules in the epidermis decreased in the HPB-treated depigmented skin in comparison with the only UV-treated and untreated skin (Figs. 4A, B, D, E and Figs. 5A, B, D, E). Melanocytes and melanin granules were almost undetectable in brown guinea pig skin (Figs. 4B, E). On the other hand, when re-pigmentation was achieved, melanocytes in the basal layer and melanin granules in the epidermis were recovered (Figs. 4C, F and Figs. 5C, F).

Effect on keratinocytes

Topical application of HPB marginally induced epidermal thickening in brown and black guinea pigs (Figs. 4G-I and Figs. 5G-I). However, marked inflammatory mononuclear cell infiltration was not observed in the HPB-treated skin. HPB-treated skin and untreated skin had almost the same number of keratinocyte layers, but different sizes of keratinocytes. Epidermal thickening returned to normal

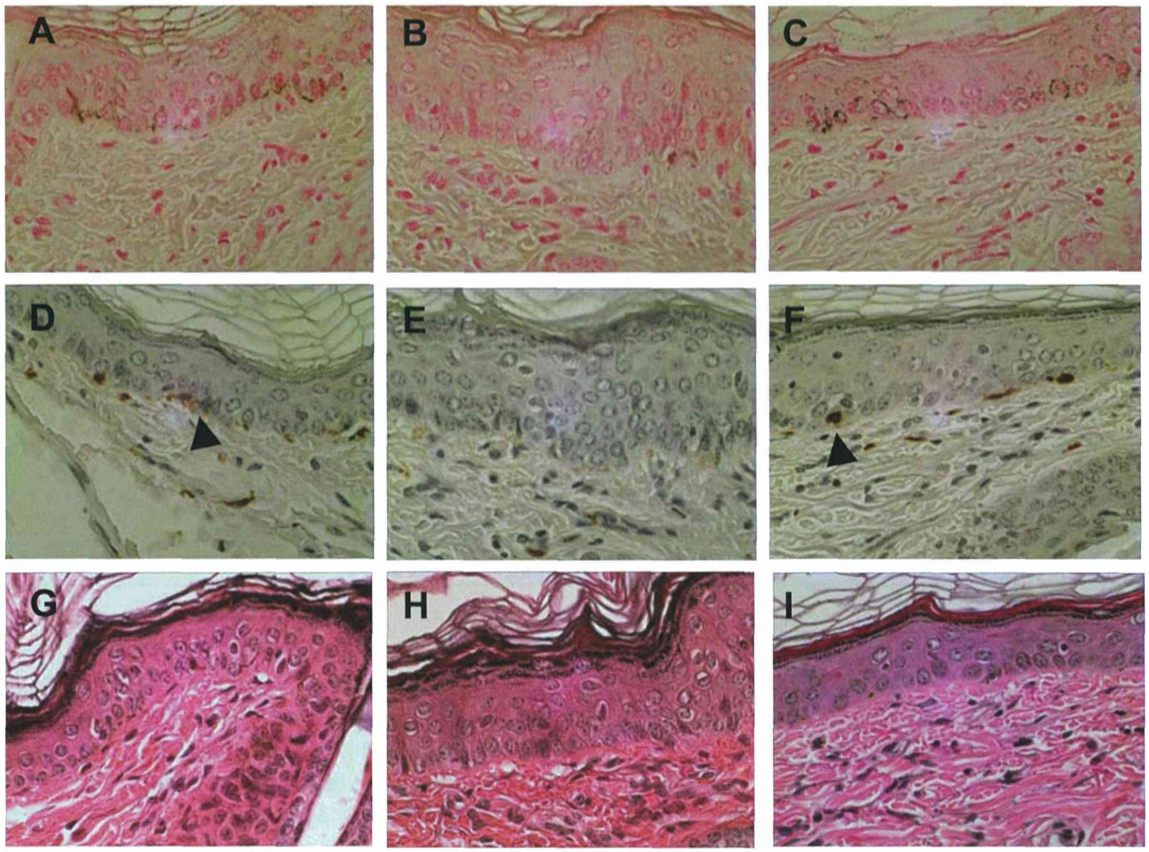

Fig. 4. Localization of melanocytes and melanin granules in the epidermis of brown guinea pigs. (A-C) Fontana-Masson silver stain, (D-F) immunohistochemical staining for S-100, (G-I) hematoxylin and eosin stains. Vertical paraffin sections were prepared from only UV-treated skin on day 41 (A, D, G), from UV+HPB-treated skin on day 41 (B, E, H), and from HPB-withdrawn skin after UV+HPB-treatment on day 78 (C, F, I) of brown guinea pigs. Brown and black dots indicate melanin granules (A-C, G-I). Black arrowheads indicate S-100 positive melanocytes (D, F). Original magnification x 20.

on day 78, when re-pigmentation was observed.

DISCUSSION

The present study shows that the frequent continuous topical application (3 times per day) of a high concentration (30%) of HPB to the backs of brown and black guinea pigs induces significant and patchy skin depigmentation. This was caused by the reduction of dopa-positive and S-100-positive epidermal melanocytes as well as a decrease in the amount of melanin granules. However, these epidermal changes spontaneously recover after withdrawing the application of HPB. The results in brown guinea pigs were similar to those in the black guinea pig.

These results are similar to previous reports where 1-5% 4-isopropylcatechol induced a potent skin depigmentation of the ear and dorsal skin of black guinea pigs (Bleehen *et al.*, 1968), or where topical application of 2% or 5% HQ to the skin induced a more potent depigmentation in black guinea pigs (Jimbow *et al.*, 1974) or where 5% PHQ induced more skin depigmentation on the backs of JY-4 black guinea pigs (Tayama and Takahama, 2002). For the chemicals reported in the literature, a concentration of 1-5% was reported to induce potent skin depigmentation by application once per day. Yet, for HPB, obvious skin depigmentation could not be achieved unless a 30% concentration was topically applied 3 times per day. On the other hand, a 10% concentration of HPB topically applied 1 time per day did not induce skin depigmentation compared to baseline skin levels (data not shown). Skin depigmentation caused by the toxicity of epidermal melanocytes is a common phenomenon among these

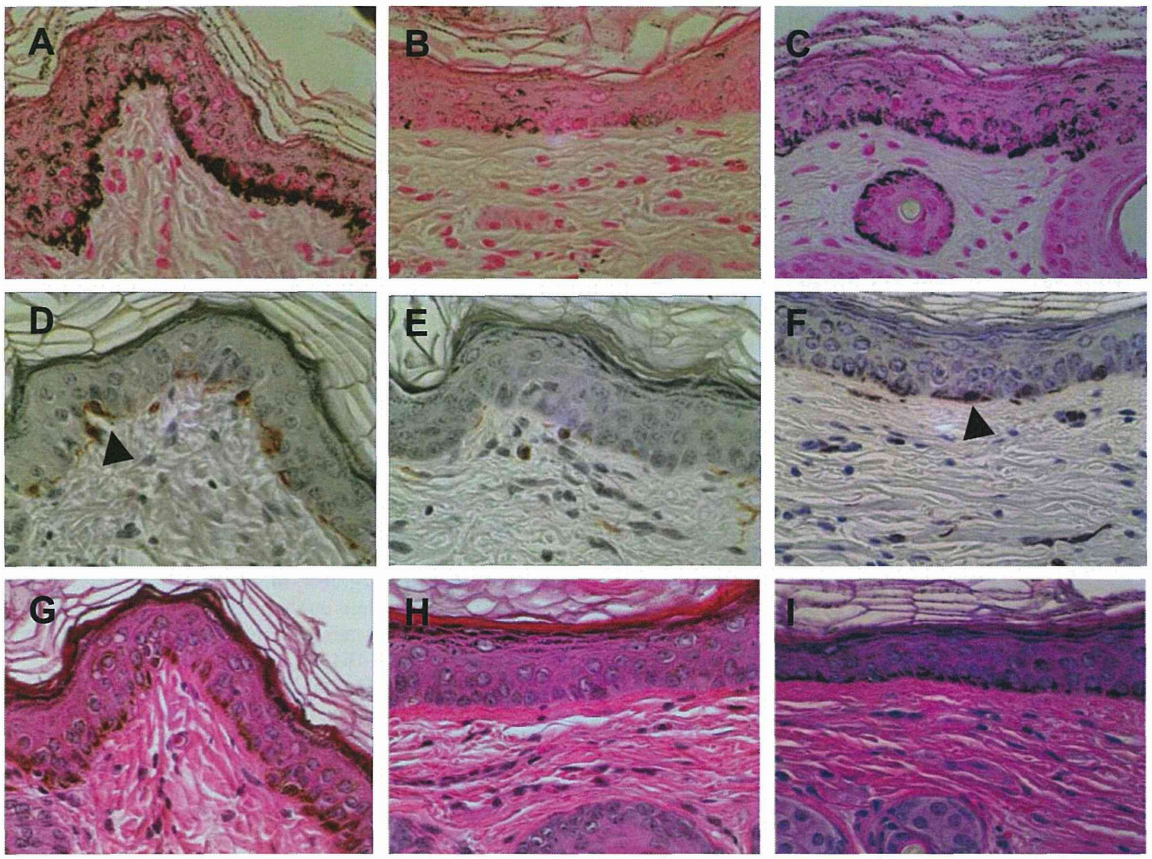

Fig. 5. Localization of melanocytes and melanin granules in the epidermis of a black guinea pig. (A-C) Fontana-Masson silver stain, (D-F) immunohistochemical staining for S-100, (G-I) hematoxylin and eosin stain. Vertical paraffin sections were prepared from untreated skin (A, D, G), from HPB-treated skin on day 22 (B, E, H) and from HPB-withdrawn skin after HPB-treatment on day 92 (C, F, I) of a black guinea pig. Brown and black dots indicate melanin granules (A-C, G-I). Black arrowheads indicate S-100 positive melanocytes (D, F). Original magnification x 20.

reports and our findings. Alkyl phenols, such as monobenzyl ether of hydroquinone (MBEH), monomethyl ether of hydroquinone, p-tertiary amyl phenol and p-tertiary-butyl catechol, have potent depigmenting capacities (Gellin *et al.*, 1979). Common chemical features of those structures are the hydroxyl group that could bind at the 4 (or para) position and the non-polar side chains at position 1 of the aromatic ring (Bleehen *et al.*, 1968). HPB (Fig. 1) has a similar feature among the alkyl phenols mentioned, however, HPB differs by not having non-polar side chains.

HPB is similar in chemical structure to RK, in which 3 cases of occupational leukoderma have been reported in chemical factory workers (Fukuda *et al.*, 1998b). Two mechanisms have been suggested for the RK-induced depigmentation: toxicity to melanocytes and inhibition of melanogenesis (Fukuda *et al.*, 1998c; Lin *et al.*, 2011). Fukuda *et al.* (1998c) reported that the 50% growth inhibition concentration of B16 melanoma cells by RK was 0.13 mM, but that a 1 mM RK solution enhanced the tyrosine hydroxylase activity of B16 cells. On the other hand, Lin *et al.* (2011) reported that 0.6 mM RK did not show any cytotoxicity although it strongly inhibited melanogenesis in B16 cells. The mechanisms involved remain controversial.

Besides the changes elicited in melanocytes, an effect of HPB on keratinocytes was also observed. An epidermal thickening was observed in our study and was similarly reported with other depigmentation reagents (Gellin *et al.*, 1979; Jimbow *et al.*, 1974; Tayama and Takahama, 2002). Taken together, we suggest that the HPB-induced depigmentation occurs via selective melanocyte toxicity.

These chemicals also have a structural similarity to

tyrosine and may have a competitive inhibition effect with tyrosinase (Denton *et al.*, 1952). Riley (1969a, 1969b, 1970, 1971 and 1975) suggested that these chemicals are incorporated into melanogenic cells and form semiquinone free radicals, which lead to the destruction of the lipoprotein membrane, and thus cause melanocyte death. In addition, HPB may be metabolized by tyrosinase. In fact MBEH can be metabolized to a quinone form and can generate cytotoxic reactive oxygen species (van den Boorn *et al.*, 2011). Hariharan *et al.* (2010) showed that 4-tertiary butyl phenol induces apoptosis. In contrast, MBEH induces not the apoptotic but the necrotic pathway leading to melanocyte death. If HPB was to induce melanocyte necrosis, an inflammatory reaction would have been observed. In the present study, we could not detect a marked increase in inflammatory monocytic cells even when a high concentration of 30% HPB was continuously applied. Thus, we hypothesize that HPB induces melanocyte apoptosis. However, further studies will be required to reveal the detailed mechanism(s) involved.

Our results demonstrate that epidermal melanocytes in the basal layer are selectively disrupted. We also found that the depigmented skin gradually re-pigments because dermal melanocytes in the basal layer re-emerge without any treatment. Speculation regarding the mechanism(s) underlying the re-emergence of epidermal melanocytes leads to 2 possible explanations. First, this phenomenon might be attributed to the migration and differentiation of melanocyte stem cells (McSCs). McSCs in the bulge or secondary hair germ can be a reservoir, not only for follicular melanocytes required for cyclic hair pigmentation but also for epidermal re-pigmentation (Nishimura, 2011). The second possible explanation is that surrounding epidermal melanocytes might migrate to depigmented sites. The former mechanism is likely since the migration of McSCs from the bulge or secondary hair germ to the epidermis is enhanced by UV-B irradiation (Chou *et al.*, 2013).

In conclusion, we demonstrate that HPB has a depigmenting activity via its selective toxicity to epidermal melanocytes not only in black guinea pigs but also in brown guinea pigs. We further show that this depigmentation is reversible.

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• CONTRIBUTIONS TO THIS SECTION MAY NOT UNDERGO PEER REVIEW, BUT WILL BE REVIEWED BY THE EDITOR •

Allergic contact dermatitis caused by 3-*o*-ethyl-L-ascorbic acid (vitamin C ethyl)

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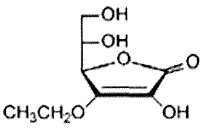
Key words: allergic contact dermatitis; cosmetics; 3-*o*-ethyl-L-ascorbic acid; skin-lightening agent; vitamin C ethyl.

Skin-lightening agents such as kojic acid, arbutin, ellagic acid, lucinol and 5,5′-dipropylbiphenyl-2,2′-diol are used in 'anti-ageing' cosmetics. Cases of allergic contact dermatitis caused by these skin-lightening agents have been reported (1, 2). Vitamin C and its derivatives have also been used in cosmetics as skin-lightening agents for a long time. Vitamin C in topical agents is poorly absorbed through the skin, and is easily oxidized after percutaneous absorption. Recently, ascorbic acid derivatives have been developed with enhanced properties. The ascorbic acid derivative 3-*o*-ethyl-L-ascorbic acid (CAS no. 86404-04-8, molecular weight 204.18; Fig. 1), also known as vitamin C ethyl, is chemically stable and is more easily absorbed through the skin than the other vitamin C derivatives. Moreover, 3-*o*-ethyl-L-ascorbic acid has skin-lightening properties. Here, we report a case of allergic contact dermatitis caused by a skin-lightening lotion containing 3-*o*-ethyl-L-ascorbic acid.



Molecular formula: $C_8H_{12}O_6$
Molecular weight: 204.18
CAS no. : 86404-04-8

Fig. 1. Chemical formula of 3-*o*-ethyl-L-ascorbic acid (vitamin C ethyl).

Case Report

A 49-year-old female presented with a 6-month history of periocular erythema and perioral swelling. She had applied a skin-lightening lotion to the face every summer for the past 6 years. In the previous summer, an itchy erythematous rash appeared on her face. She stopped using the lotion, and consulted a dermatologist. She received a 3-day course of mequitazine (6 mg daily), betamethasone (1 mg daily), and teprenone (150 mg daily), as well as topical corticosteriod ointments (prednisolone acetate for the periocular skin lesion, and hydrocortisone butyrate for the face).

We performed patch tests with the patient's personal cosmetics and cosmetic allergens at our hospital outpatient clinic. Finn Chambers® (Smart Practice, Phoenix, AZ, USA) mounted on Scanpor® tape (Norgesplaster AS, Vennesla, Norway) were applied to the upper back for 2 days, and the reactions were read on D2, D3 and D7 according to International Contact Dermatitis Research Group criteria. A positive reaction to the skin-lightening lotion (neat) was observed (D3, +; D7, +), and the repeated open application test (ROAT) resulted in an itchy erythema. A second patch test with the skin lotion ingredients was performed, and gave positive reactions to 3-*o*-ethyl-L-ascorbic acid in 5% pet. (D3, +; D7, +), 1% pet. (D3, +; D7, +), 0.5% pet. (D3, +; D7, +), 0.1% pet. (D3, +; D7, +), and 0.05% pet. (D3, +; D7, +), but not in 0.01% pet. From the patch test findings, the patient was diagnosed with allergic contact dermatitis caused by 3-*o*-ethyl-L-ascorbic acid. The minimum positive concentration of 3-*o*-ethyl-L-ascorbic acid was 0.05% pet. We examined ascorbyl tetraisopalmitate (CAS no. 183476-82-6) 1% pet. and magnesium ascorbyl phosphate (CAS no. 114040-31-2) 1% pet. as vitamin C derivatives. We

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performed patch test using the same substances on the inner side of the upper arms of three healthy controls. They showed negative reactions.

Discussion

Vitamin C and its derivatives have been deemed to be safe for use in cosmetics. The Cosmetic Ingredient Review reported that L-ascorbic acid, calcium ascorbate, magnesium ascorbyl phosphate, sodium ascorbate and sodium ascorbyl phosphate are safe for use in cosmetic products (3). Despite the cosmetic safety of vitamin C derivatives such as magnesium L-ascorbyl 2-phosphate and ascorbic acid 2-glucoside, they lack antioxidant properties, and rapidly lose their effectiveness. New vitamin C derivatives have been produced with enhanced stability. 3-*o*-Ethyl-L-ascorbic acid is a new vitamin C derivative that is more stable, with preservation of its vitamin C activity (4), and is currently used in cosmetics as a skin-lightening agent. Cases of allergic contact dermatitis caused by L-ascorbic acid-containing and ascorbyl tetraisopalmitate-containing creams have been reported (5, 6), as have cases of delayed-type allergy caused by oral ingestion of vitamin C (7), but allergic contact dermatitis caused by 3-*o*-ethyl-L-ascorbic acid has not been reported to date.

In this report, we describe a case of allergic contact dermatitis caused by a skin-lightening lotion containing 3-*o*-ethyl-L-ascorbic acid. The maximum concentration of 3-*o*-ethyl-L-ascorbic acid in the skin lotion is 2%. The patient had a positive patch test reaction to the skin lotion, and an itchy erythema and papules appeared at the ROAT application site. Patch testing with the ingredients of the skin lotion indicated that 3-*o*-ethyl-L-ascorbic acid was the causative allergen. Different concentrations (5%, 1%, 0.5%, 0.1%, 0.05%, and 0.01%) of the allergen in pet. were patch tested, and showed the minimum positive concentration to be 0.05% pet. To the best of our knowledge, our case is the first reported case of contact dermatitis caused by 3-*o*-ethyl-L-ascorbic acid.

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ロドデノール誘発性脱色素斑

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㈱協和企画

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topics

ロドデノール誘発性脱色素斑

松永佳世子*

Key words
ロドデノール誘発性脱色素斑，美白剤，化粧品，白斑

はじめに

ロドデノール（以下，RD）誘発性脱色素斑（Rhododenol-induced leukoderma）とは，RD含有化粧品を使用後，主に使用部位に生じるさまざまな程度の脱色素斑で，使用中止により一部あるいは全体に色素再生がみられることが多い[1]（**図1**）．

RD：Rhododenolは商品名で，一般名はロドデンドロール（rhododendrol），別名に4-(4-ヒドロキシフェニル)-2-ブタノールなどがある．本剤は，2008年1月，「メラニン生成を抑え，しみ，そばかすを防ぐ効果を有する」新規医薬部外品有効成分として，厚生労働省の認可を取得した．本剤を配合した化粧品を使用した人の中に色素脱失をきたした症例が複数確認された結果，2013年7月4日に株式会社カネボウ化粧品，ならびに関連会社の株式会社リサージ，株式会社リサップは本剤を含むすべての化粧品（**図2**）を自主回収に踏み切った．

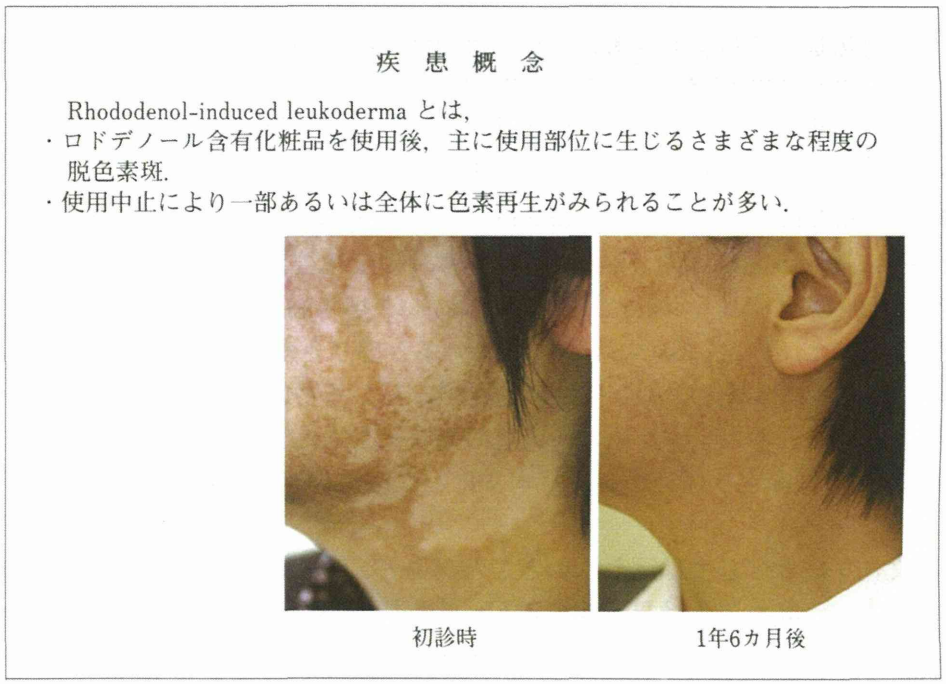

図1　RD誘発性脱色素斑の疾患概念と典型例．初診時およびRD含有化粧品中止後1年6カ月後の回復時臨床像．

日本皮膚科学会は，その責任ある立場から，症例の実態調査を行い，医療者（皮膚科医）と患者向けに正しい情報を提供し，診断と治療方法を早急に確立するために「ロドデノール含有化粧品の安全性に関する特別委員会（委員長：松永佳世子）」を2013年7月17日に発足し活動してきた．特別委員会は患者のためにFAQ[2]，医療者（皮膚科医）向けの診療の手引き[1,3]を作成し，一次，二次全

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図2　自主回収になったRD含有(配合)化粧品一覧

国疫学調査を施行し[4,5]，その実態と診断と治療に役立つ情報を提供してきた．また治療に役立てるための病態解明の研究を行い，その成果を日本皮膚科学会ホームページに掲載し改訂してきた[6,7]．

本稿では，RD脱色素斑について，これまでに得られた知見の概要を紹介する．

Ⅰ．RDの含まれる化粧品

RDの含まれた製品を図2に示す．詳細は厚生労働省[8]，カネボウ化粧品[9]のホームページを参照いただきたい．

Ⅱ．RDの構造と作用機序

1．発見と由来

カネボウ化粧品では，多くの植物由来のさまざまな天然物質について，メラニンの生成を抑える作用の有無をスクリーニングした結果，4(4-ヒドロキシフェニル)-2-ブタノールという物質に着目した．その後，詳しく調べたところ，メラニンの生成を抑える効果が非常に高いことが明らかになった．2008年には厚生労働省より，メラニンの生成を抑え，しみ，そばかすを防ぐ効能で医薬部外品有効成分として承認された．

2．“美白作用”を示す機序

皮膚のしみは，メラニン色素が皮膚へ過剰に沈着するため生じる．そのメラニンは皮膚に存在する色素細胞の中で合成されるが，メラニンの生成にもっとも重要な役割を果たすのがチロシナーゼという酵素である．メラニン生成反応は，チロシナーゼによるチロシンの酸化が出発点となり，その先の反応過程へと進むが，チロシナーゼはこのメラニン生成過程における律速酵素で，この反応がおこらなければ，メラニンはまったく生成されない．近年，メラニンの生成にはチロシナーゼの

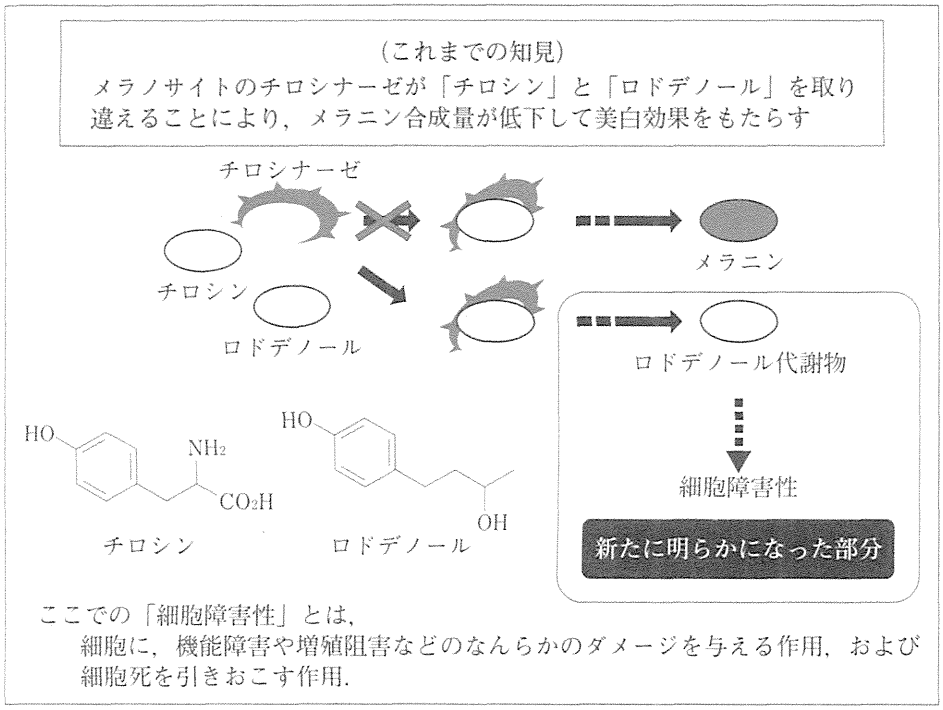

図3　RDの「美白」作用のメカニズムと細胞障害性

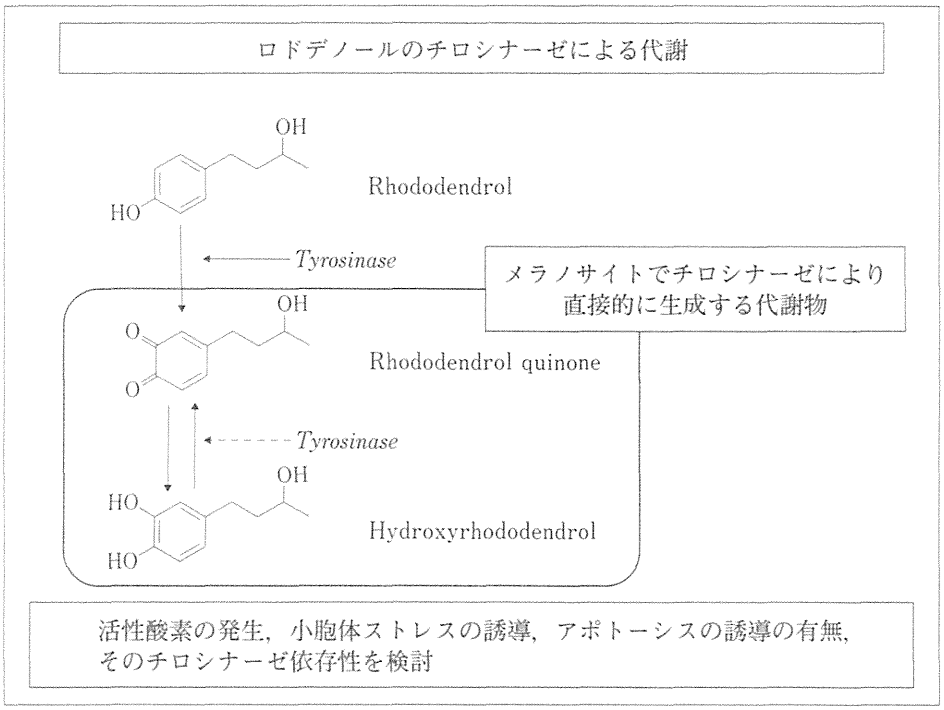

図4　RDのチロジナーゼによる代謝

みならず，2種類のチロシナーゼ関連蛋白質も重要な役割を果たすことがわかっている．RDはチロシナーゼおよび2種類のチロシナーゼ関連蛋白質の働きを抑制することにより，メラニン生成を抑制する．

そのメカニズムの詳細は不明ながらも以下のように考えられている．RDはメラニン生成の出発材料であるチロシンとその構造が類似しているため，本来はチロシンが結合するべきチロシナーゼの活性中心に結合する．その結果として，チロシナーゼに本来の反応基質であるチロシンが結合できなくなり，メラニン生成反応が進行せず，メラニンの生成が減少することになる．こういった酵素阻害様式を拮抗阻害という．拮抗阻害作用は，チロシンとRDの相対的な濃度によって決定されるので，RDの濃度が減少，つまり使用を中止すれば，その効果は消えるものと考えられる．また，脱色素斑部ではメラノサイトの減少が認められることから，メラノサイトへのなんらかの障害作用もあることが推測されている（図3～5）．

Ⅲ．RD脱色素斑の臨床症状の特徴および前駆症状

①RDを含有する化粧品を使用後2カ月から3年経ち，不完全脱色素斑*が顔面，頸部，手背，腕に分布する．脱色素斑はまだらなことが多く，色素脱失の程度はさまざまである．色素脱失の程度が軽く，境界も不明瞭で一

見して目立たなくても，よくみると脱色素斑を生じていることもある．一方で境界明瞭な完全脱色素斑*に移行したと考えられる症例もみられる．なお，色素斑が完全か不完全かよく区別できない場合も，ダーモスコープで観察すると脱色素斑部において毛は色が付いている場合が多い．

②化粧品の塗布部位に痒みを伴う紅斑を認めることがある．炎症後に脱色素斑が生じる例や，脱色素斑と正常部の境界に炎症を伴う炎症型白斑*を呈する症例もある．まったく炎症を伴わない症例もある．炎症を伴う症例，伴わない症例ともにRDによるパッチテストが陽性の症例がある(図6)．なお炎症を伴う症例群のほうが，より高いパッチテスト陽性率を示す．

③RD含有化粧品の使用を中止後，6カ月くらいで色素再生を認める例が多い．完全脱色素斑から不完全脱色素斑を経て回復する場合や，毛包一致性の点状色素再生を認める場合がある．

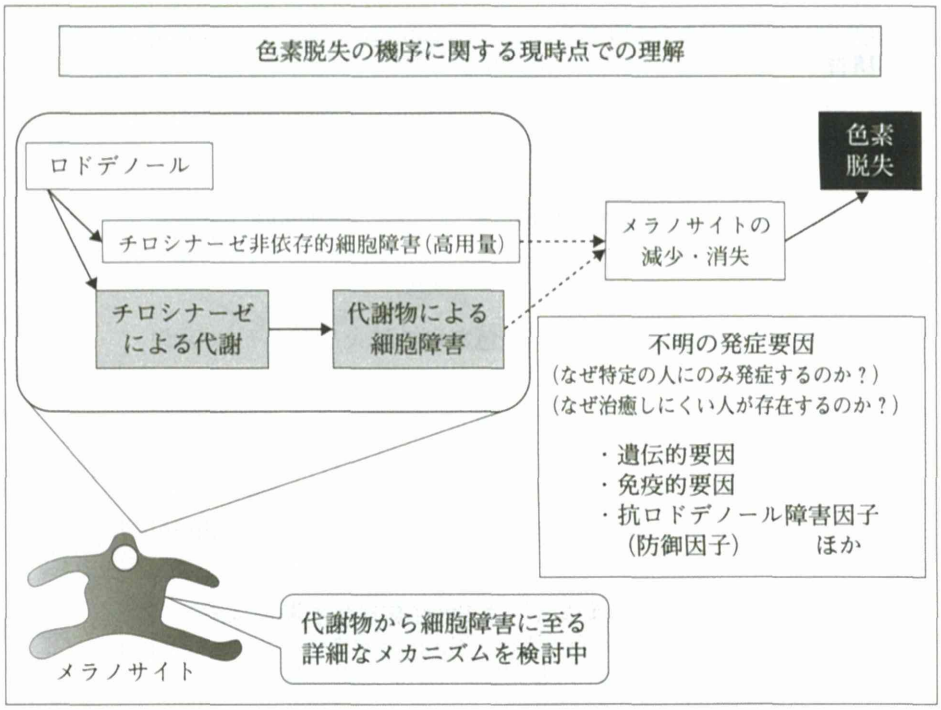

図5　RDによる色素脱失の機序に関する現時点での理解

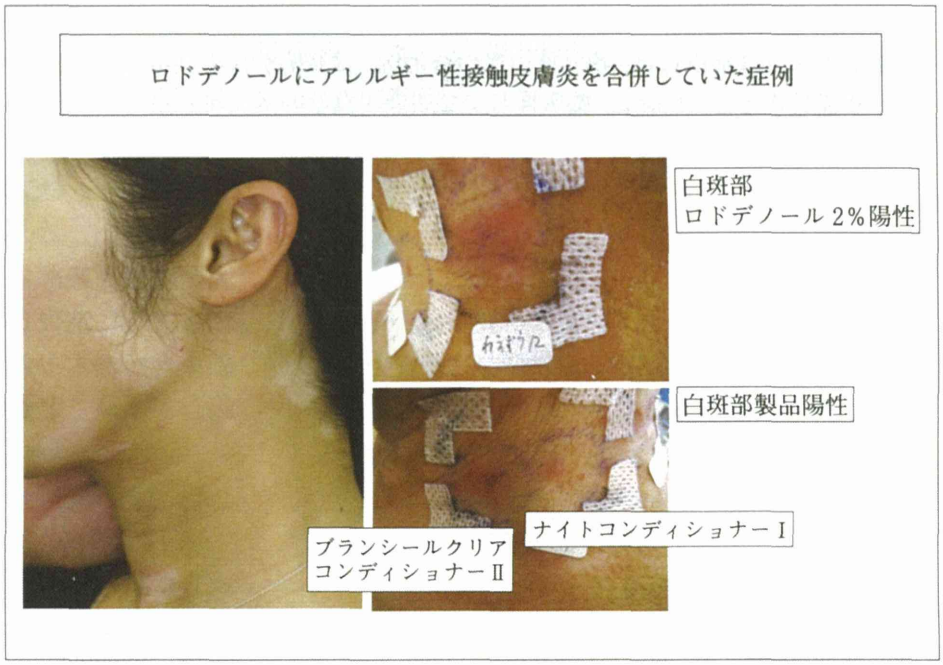

図6　RDにアレルギー性接触皮膚炎を合併していた症例の臨床像とパッチテスト陽性所見

④回復過程に色素増強(temporal excess) repigmentationを認める例がある．一過性の色素増強は軽快することが多い．

⑤大半が女性であるが，家族に勧められて使用した男性例もあった．

【用語の定義】

＊完全脱色素斑：ほぼ，あるいは完全にメラニン色素が欠如して，健常色を喪失し，白色調を呈する脱

表　RD誘発性脱素斑の診断基準

必須項目

1. ロドデノール含有化粧品を使用していた.
   注)患者申告 購入履歴, 回収記録を根拠に判断する.
2. ロドデノール含有化粧品を使用する前には脱色素斑がなく, 使用後, 使用した部位におおむね一致して生じた, 完全ないし不完全脱色素斑がある.

小項目

1. 使用中止により(必須項目 2. の)脱色素斑の拡大がおよそ1カ月以内に停止した.
2. 使用中止により(必須項目 2. の)脱色素斑の少なくとも一部に色素が再生した.
   注)写真や診療録, ダーモスコピー所見などの記録を参照し, 医師が視診により重症度判定基準を用いて判定する.

判定

・必須項目2項目と小項目の少なくとも1項目を満たす場合は確実例とする.
・必須項目2項目を満たすが, 小項目の1, 2ともに満たさない場合はその時点では疑い例とする.
・疑い例については引き続き注意深く色素再生の有無を経過観察することが望ましい.
   注)このような症例には, 尋常性白斑の合併例, 誘発例が含まれる可能性がある. しかし, 臨床像および病理組織学的所見から尋常性白斑とロドデノール誘発性脱色素斑を鑑別することは困難な場合があり, 診断には細心の注意が必要である.

色素斑.

＊不完全脱色素斑：メラニン色素が減少し, 健常色に比し白色調を呈するが, 健常色の完全喪失には至っていない脱色素斑. ただし, 両者の区別は視診で行うものとし, 混在していること, 連続していること, 時期によって変動することがある.

＊炎症型白斑：白斑の辺縁に紅斑や浸潤を伴う脱色素斑.

Ⅳ. 臨床分類

1. 完全脱色素斑優位型

完全脱色素斑のみ, もしくは完全脱色素斑優位(脱色素斑面積全体のうち6割以上が完全脱色素斑).

2. 完全・不完全脱色素斑混合型

完全脱色素斑と不完全脱色素斑優位がほぼ同じ割合で混在する.

3. 不完全脱色素斑優位型

不完全脱色素斑のみ, もしくは不完全脱色素斑優位(脱色素斑面積全体のうち6割以上が不完全脱色素斑).

Ⅴ. 病理組織像

生検組織の結果, 色素細胞が消失している症例, 色素細胞が減少している症例, 炎症細胞浸潤を伴っている症例や真皮浅層にメラノファージが散見されるだけの症例など, 臨床像と同じく病理組織像も多彩である. 尋常性白斑との鑑別について, ①毛嚢周囲に細胞浸潤がみられる, ②メラノファージが大多数の症例にみられる, の2点が尋常性白斑との区別の参考となる. メラノファージが認められないものは現時点では尋常性白斑の可能性が高いとの結論となっている. また, 尋常性白斑においては, 多くの場合, 完全脱色素斑部ではメラノサイトの完全な消失を認めるのに比して, 本疾患では臨床的に完全脱色素斑部でも, メラノサイトの減少はあっても完全に消失している症例は少数であり, 毛嚢部を含め標本上のいずれかの部位に残存を認める場合が多いことが明らかになっている(図7).

Ⅵ. 診　断

以上を踏まえて, RD脱色素斑の診断基準を作成した(表)[3].

Ⅶ. 鑑別診断

尋常性白斑との鑑別は尋常性白斑診療ガイドラインを参考に行う. 分節型は当該化粧品使用部位と一致しないので否定できる. 汎発型は区別がむずかしい場合がある. 脱色素斑の発症時期が当該化粧品の使用後であるか, 使用部位に一致しているか, 臨床的および病理組織学的に完全脱色素斑

であるか，甲状腺機能，膠原病，糖尿病，Addison病，脱毛の有無等も，必要に応じて確認し，除外する必要がある．

二次調査の結果，当該化粧品の中止により72％の患者で使用していた部位に色素の再生がみられている．したがって，経過観察により色素再生が認められれば，RD誘発性脱色素斑の可能性が高いと考えられる．

Ⅷ. 患者数・発生頻度

2014年11月28日現在，RDで脱色素斑を生じた症例は19,370名にのぼっており，そのうち9,243名(47.7％)が完治・ほぼ回復したとカネボウ化粧品の調査で報告されている[9]．RD含有化粧品使用者が80万人と推定されているので，使用者の約2.4％が発症したことになる．

Ⅸ. パッチテスト陽性例の頻度

これまでに予備試験を含めて52施設199例のパッチテスト結果が収集されている．そのうち，48時間後のみの判定しか記載がなく，陽性の判断が困難であった14例を除く185例につき解析した．その結果，①2％RD白色ワセリン基剤陽性は全体の13.5％(25/185)，②炎症あり症例の陽性率は20.0％(20/100)，③炎症なし症例の陽性率は6.8％(5/74)，④炎症の有無が不明の症例での陽性率は0％(0/11)，⑤1週間後判定時に2％pet貼布部(健常皮膚部)に「白斑出現」

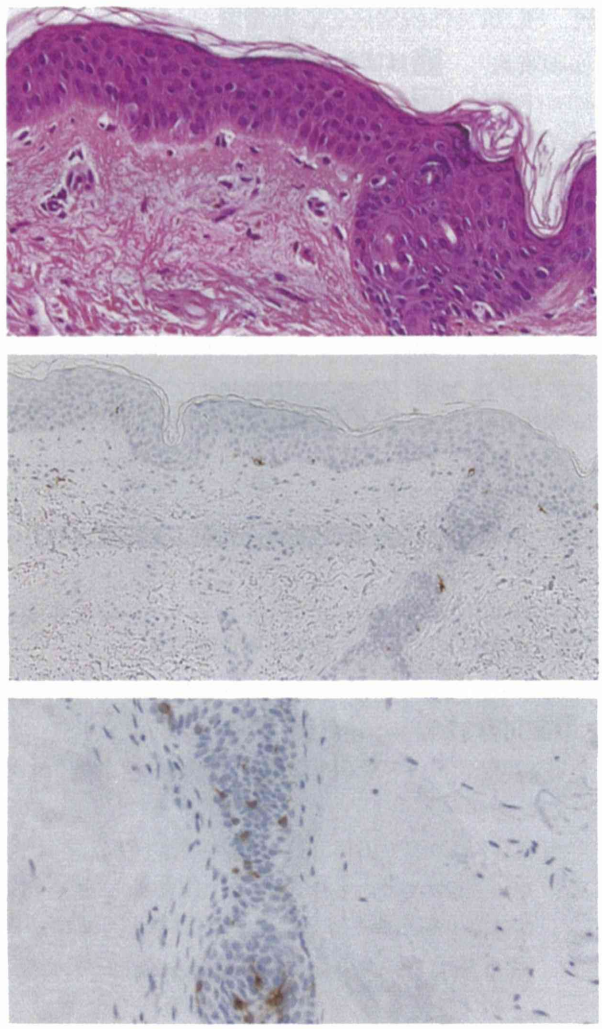

図7　RD誘発性脱色素斑症例の脱色素斑部の病理組織像

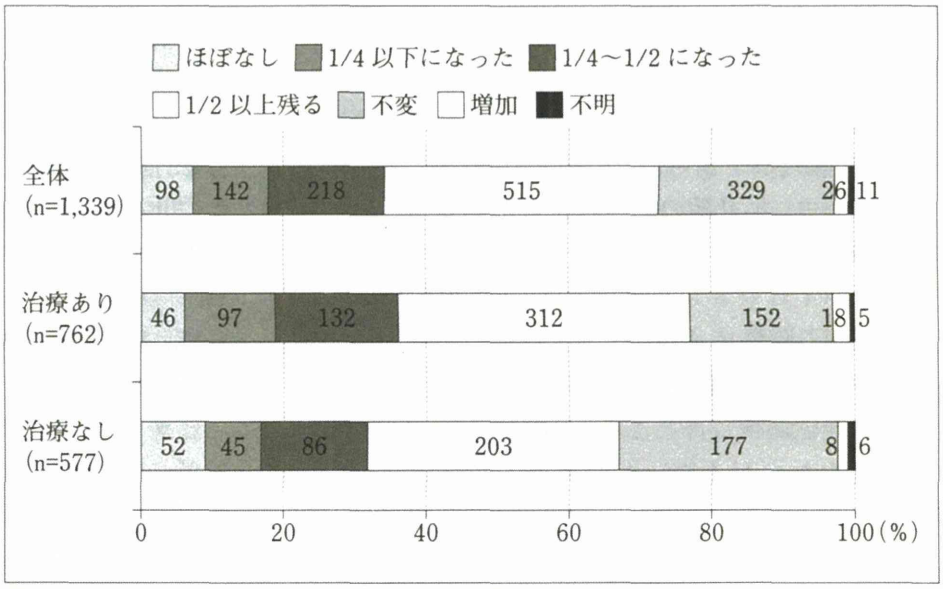

図8　治療の有無と脱色素斑面積の変化(全国二次調査より)

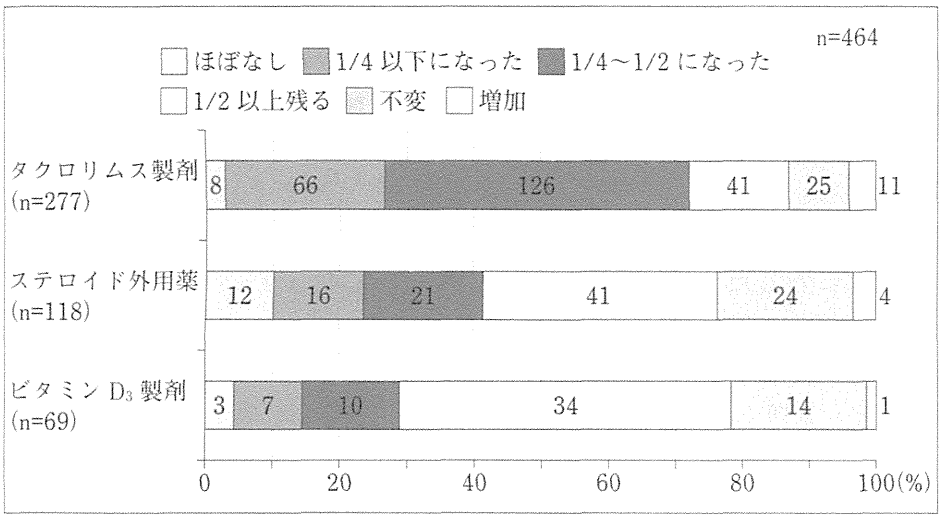

図9　外用薬と脱色素斑面積(全国二次調査より)

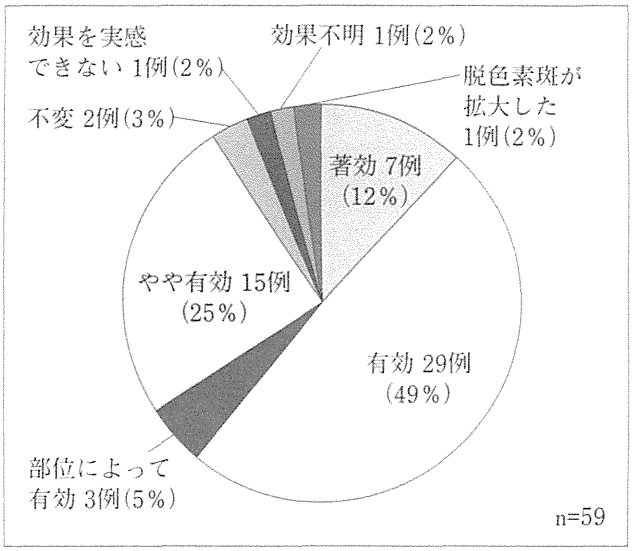

図10　紫外線治療の効果

と記載ある症例が1例，「うっすら白いか」と記載ある症例が1例であった．これら2例は別の施設からの報告で，集計数の約1%(2/199)になる．

2%RD白色ワセリン基剤を全国に配布する前に行ったパイロットスタディでは，5%RD白色ワセリン基剤の陽性率も検討した．73例に貼布し，2%が陰性であった54例のうち26例に5%RD白色ワセリン基剤を貼布したところ，4例(15.4%)に陽性反応を認めた．パイロットスタディーではRDの光線過敏についても検討し，52例に光パッチテストを施行した．その結果，UVA照射により反応が増強した症例が1例あった．

X. 予　後

2014年1月に行った全国二次調査では，「経過観察あり」と回答のあった1,399例中，7%は「脱色素斑がほぼなくなった」，11%は「1/4以下になった」，16%は「1/4～1/2になった」，38%が「1/2以上残る」，25%は「不変」，2%が「増加」，0.9%が「不明」，0.1%が「評価困難」であった(図8)．自主回収から半年後の調査であったため，中止して半年経過した患者さんが最多であったが，118例が，1年以上前に当該化粧品を中止しており，このうち6割以上は医療機関で経過観察中に脱色素斑面積が縮小傾向ありと回答している．全国二次調査時点では，まだ中止後1年を経過していない症例が多く，これからも引き続き経過観察をする必要がある．治療の有無で比較してみると，なんらかの治療を受けた方の77%が，また，治療せずに経過をみている方でも67%が脱色素斑面積の縮小傾向を示していた．

XI. 治療と経過

1. 治療の基本

まずは当該化粧品を中止したうえで，遮光をしっかり行い，経過観察をすることが第一選択である．前述の全国二次調査の結果，経過観察のあった1,399例中「治療あり」は57%，「治療なし」は43%であった．無治療で経過をみている症例でも67%が回復傾向を示し，なんらかの治療を受けている方では77%が回復傾向を示していた．

2. 治療の内容

ビタミンC，トラネキサム酸，ビタミンE，アレルギー薬などの内服治療と，タクロリムス軟膏，ステロイド外用薬，ビタミンD_3外用薬などによる外用治療，ならびに紫外線治療等が行われている．

3. 外用薬治療と経過

タクロリムス外用薬単独使用群，ステロイド外用薬単独使用群，ビタミンD_3外用薬単独使用群の3群で比較すると，タクロリムス軟膏単独使用群が，ほかの外用薬単独使用群や，治療なしと回答した群に比べて，脱色素斑面積の評価（ほぼなくなった；1/4以下になった；1/4～2/1になった；1/2以上残る；不変；増加）で，「半分以下に縮小している」と回答した割合が高く（**図9**），色素増強も含めた総合評価（治癒；かなり軽快；軽快；やや軽快；不変；増悪）でみると，ステロイド外用薬単独使用群で「軽快以上」の評価であった症例の割合が高くなっていた．ビタミンD_3外用薬の効果は，症例数も少なくはっきりした有効性は確認できていないが，タクロリムス軟膏やステロイド軟膏を用いにくい症例では試みてもよいと考えられている．

4. 紫外線治療と経過

「紫外線治療あり」と回答のあった75例に対して2014年5月に治療内容や効果を再度調査したところ（集計対象66例），効果について回答のあった60例中，著効からやや有効と回答したのは56例（84.8%）であった．化粧品中止の効果と判別がむずかしいという意見も複数あり，紫外線治療により1例は「脱色素斑面積が拡大した」，2例は「不変」，1例は「軽快しているが紫外線治療の効果であると実感できない」と合計4例（6.0%）が回答している．紫外線治療は，ほかの治療で軽快がみられない症例には試みてよい治療方法と思われる（**図10**）．

ただし，紫外線治療の効果はあったが「刺激が出現しやすく照射量を低めに設定した」，「脱色素斑周囲に色素増強をきたしたので中止した」という回答もあり，低容量の紫外線照射から始め，周囲の健常皮膚の遮光に十分注意して行うことが必要である．

5. 治療のまとめ

以上の全国二次調査の結果から，原因となった化粧品使用中止後一定の期間（6カ月程度）が経過しても脱色素斑の改善がみられない部位には，通常の尋常性白斑の治療が有効と思われる．なお，塗布部位や症状によっても治療法の選択は変わってくるので，経過観察以外に，これらの治療の選択は主治医の判断が尊重される．治療と経過についても，三次調査でさらに検討を行う予定である[7]．ヒトの皮膚モデルマウスにRDを外用したところ患者でみられた脱色素斑と同様な脱色素斑が生じることが明らかになった．

おわりに

現在までに，脱色素斑が改善している症例が多いが，一部では，難治の症例もある．RD誘発性脱色素斑の発症機序については，RDの代謝，作用など化学物質からの解明，なぜ一部の人にだけ，まだらに脱色素斑が生じたのかという個体側の要因等を解明すべきであるが，病態はまだ十分解明できていない．現在，ゲノム解析を含む研究が進んでおり，今後，さらに病態の解明がなされることと考える．

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