addition of rhododendrol using the fluorescence probe 2′,7′-dichlorofluorescein diacetate (CM-H_2DCFDA) (data not shown), antioxidants were observed not to rescue NHEMs from rhododendrol-induced cell viability suppression (data not shown), which clearly suggests little or no involvement of oxidative stress in the rhododendrol-induced melanocyte toxicity. These findings encouraged us to hypothesize that the rhododendrol-induced white blotching might be predominantly caused by one or more toxic metabolites derived from rhododendrol, which would be consistent with studies that suggested the involvement of tyrosinase-catalyzed metabolites with melanocyte toxicity [41,42]. Analysis using LC–MS/MS demonstrated the generation of hydroxyl-rhododendrol with a retention time around 21 min only in NHEMs with a higher activity of tyrosinase (Fig. 3). This generation after exposure to rhododendrol for 1 h was observed to be higher than that for 3 h. The incubation of rhododendrol with mushroom tyrosinase confirmed that this generation of a hydroxyl-metabolite was catalyzed by tyrosinase in a dose-dependent manner (Fig. 4).

3.3. Rhododendrol is immediately converted to a highly toxic hydroxyl-metabolite that damages melanocytes

To further examine the practical effect of hydroxyl-rhododendrol to diminish the viability of NHEMs, a hydroxyl-rhododendrol

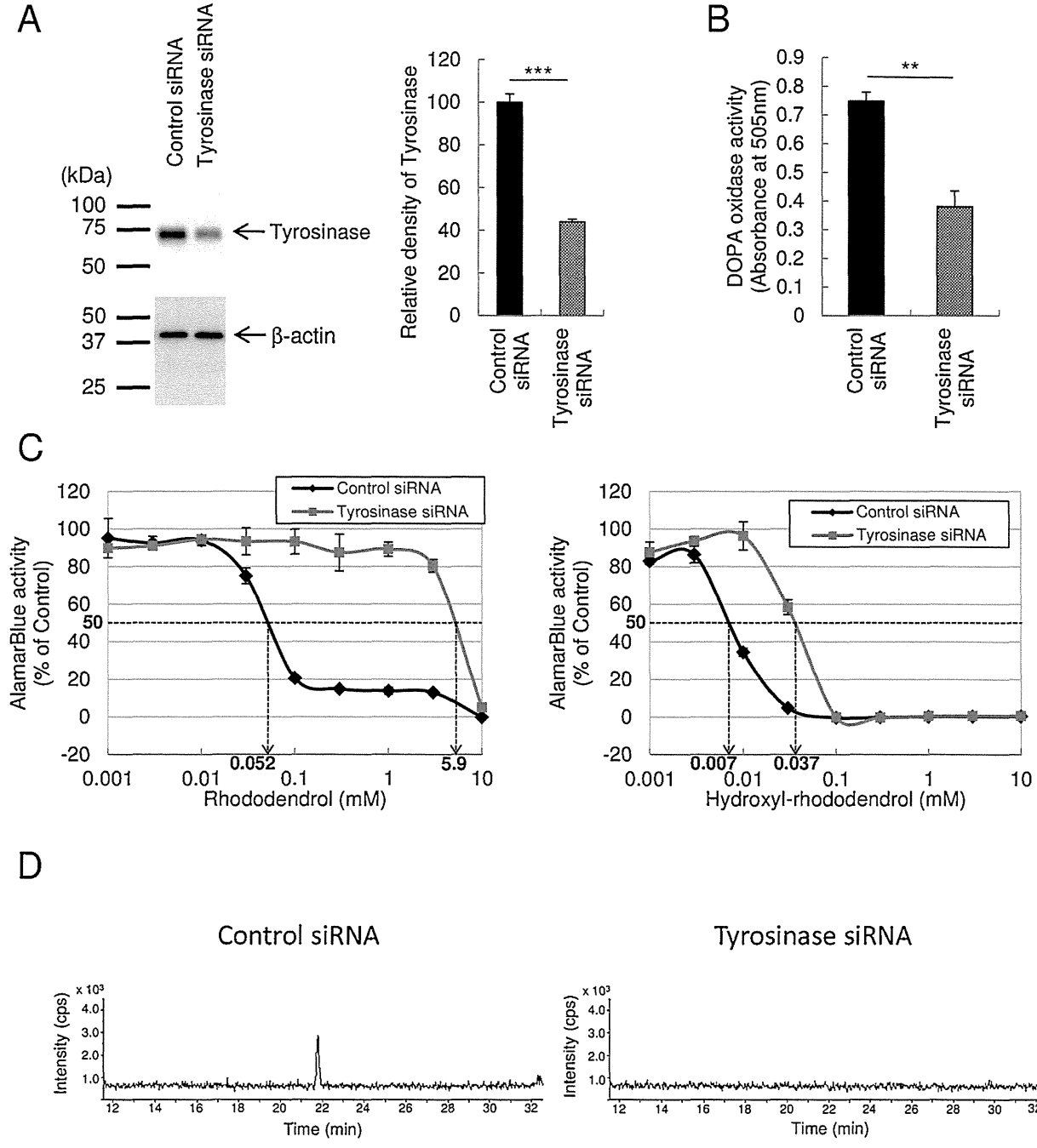

Fig. 5. *Effect of suppressing tyrosinase expression/activity on rhododendrol- and its hydroxyl-metabolite-induced melanocyte toxicity.* (A) Western blotting analysis of tyrosinase in melanocytes treated with an siRNA specific for *tyrosinase* or with a nonspecific control siRNA; values represent means + SD from 4 samples. *** $p < 0.001$ (Student's *t*-test). (B) DOPA oxidase activity of melanocytes treated with siRNAs as described above; values represent means + SD from 4 samples. ** $p < 0.01$ (Student's *t*-test). (C) Cell viability to rhododendrol or hydroxyl-rhododendrol of melanocytes treated with siRNAs as described above. (D) Detection of hydroxyl-rhododendrol in melanocytes treated with siRNAs as described above assessed using LC–MS/MS.

Table 2
IC_{50} values of cell viabilities to rhododendrol among melanocytes treated with gradually increasing amounts of a siRNA specific to *tyrosinase*.

	1	2	3	4	5	6	7	8
Concentration of tyrosinase siRNA (nM)	25.0	5.0	3.3	2.5	1.7	0.8	0.4	0
Concentration of control siRNA (nM)	0	20.0	21.7	22.5	23.3	24.2	24.6	25.0
DOPA oxidase activity (absorbance at 505 nm)	0.37 ± 0 02	0.42 ± 0.01	0.47 ± 0.02	0.47 ± 0.02	0.54 ± 0.01	0.63 ± 0.02	0.86 ± 0.23	1.02 ± 0.19
IC_{50} of AlamarBlue activity with rhododendrol (mM)	6.47	6.54	6.85	6.87	6.47	6.32	2.98	0.32

metabolite was synthesized followed by an evaluation to compare it with the toxicity of rhododendrol. The IC_{50} values of melanocyte viability tolerant to hydroxyl-rhododendrol were approximately 20 μM and 60–70 μM in NHEMs with higher and lower tyrosinase activities, respectively (Table 1). These data strongly suggested that those IC_{50} values were dramatically decreased by approximately 50–100 times compared to rhododendrol, regardless of whether NHEMs had higher tyrosinase activity or not, and that NHEMs with higher tyrosinase activity persistently showed lower IC_{50} values, indicating hydroxyl-rhododendrol to be a major causative compound that impairs NHEMs.

3.4. Suppression of tyrosinase expression rescues NHEMs treated with rhododendrol or hydroxyl-rhododendrol

To directly evaluate the contribution of tyrosinase-catalyzed hydroxyl-rhododendrol to melanocyte toxicity that could result in skin depigmentation, NHEMs treated with an siRNA specific for *tyrosinase* were incubated with different concentrations of rhododendrol or hydroxyl-rhododendrol. Because of the presence of many melanosomes containing active tyrosinase in melanocytes, the efficacy of the knockdown of *tyrosinase* expression and activity was demonstrated to be consistently reduced by approximately 50% using Western blotting and DOPA oxidase activity analyses (Fig. 5A and B). Even though there was not a complete suppression of tyrosinase activity, these siRNA-treated cells allowed us to use this approach to further determine the mechanism involved. The IC_{50} value of melanocyte viability tolerance to rhododendrol was shown to be about 50 μM in NHEMs treated with a nonspecific control siRNA, whereas that number in NHEMs treated with the siRNA specific to *tyrosinase* was about 6 mM, revealing that tyrosinase markedly augmented the cytotoxicity of rhododendrol by approximately 50–100 times (Fig. 5C). In agreement with this, LC–MS/MS analysis confirmed that treatment of NHEMs with the *tyrosinase*-specific siRNA abolished the peak of the hydroxyl-rhododendrol metabolite (Fig. 5D). Interestingly, the IC_{50} value of the viability to highly toxic hydroxyl-rhododendrol was increased by about 5 times when tyrosinase activity was inhibited by 50%, which suggests that other substantial factors might enhance melanocyte damage after the hydroxyl conversion.

3.5. A certain threshold of tyrosinase activity determines melanocyte fate

Our observation of cell damage only in NHEMs with higher tyrosinase activity encouraged us to hypothesize that there is a critical threshold of tyrosinase activity, and consequently in the ability to produce the highly toxic hydroxyl-rhododendrol metabolite. Treatment of NHEMs with gradually increased amounts of *tyrosinase*-specific siRNA suggested that tyrosinase activity exceeding a certain threshold level determined melanocyte toxicity by producing a minimal amount of the highly toxic metabolite and that tyrosinase activity above that threshold harmed NHEMs in a dose-dependent manner (Table 2, Fig. 6). Those results confirm the existence of a threshold of tyrosinase activity in NHEMs that determines whether they are tolerant or sensitive to rhododendrol.

4. Discussion

In order to satisfy the desire of consumers to brighten/lighten their skin, many active materials have been developed parallel to the findings of the precise mechanisms involved in regulating cutaneous pigmentation. Although all brightening materials have been developed and introduced to provide attractive skin care products, especially for women all over the world, some unanticipated events have caused skin problems, including the induction of white blotching. It is still unclear why only some people suffer from such skin problems and why diverse symptoms are present. While the mechanisms underlying the depigmentation caused by some phenols and catechols, such as hydroquinone, monobenzyl ether hydroquinone and/or 4-tertiary butyl phenol, have been investigated in contrast to many other depigmentation-

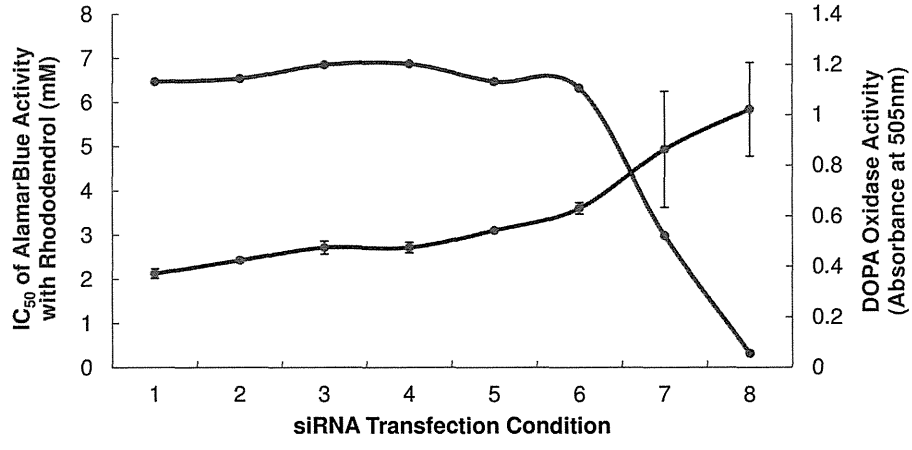

Fig. 6. *A threshold of tyrosinase activity determines melanocyte tolerance to rhododendrol in a tyrosinase-activity-dependent manner.* Melanocytes were treated with gradually increasing amounts of siRNA specific to tyrosinase, followed by characterization of the relationship between their DOPA oxidase activity and their tolerance to rhododendrol.

inducible chemicals with a poorly investigated etiology [41–45], those limited studies encouraged us to hypothesize that tyrosinase-catalyzed metabolites and/or oxidative stress are predominantly involved in the melanocyte damage that results in the skin depigmentation. In this study, the results highlight that several types of NHEMs derived from donors of different ethnic backgrounds are divided into two groups according to their tolerance/sensitivity to rhododendrol. Further, the results show that the cytotoxicity of hydroxyl-rhododendrol catalyzed by tyrosinase rather than the generation of ROS and oxidative stress is one of the causes for the reduced melanocyte viability following treatment with rhododendrol. Although most of their tolerance was dependent on their lower tyrosinase activity, one NHEM line with a higher tyrosinase activity was tolerant to rhododendrol. These findings are strongly considered to be related to one of the causes of the skin depigmentation following the use of rhododendrol-containing brightening cosmetics.

One important issue addressed in the current study is how rhododendrol causes the melanocyte toxicity. Our data demonstrate that the IC_{50} value of rhododendrol for melanocyte viability was dramatically decreased by approximately 50–100 times when rhododendrol was catalyzed to hydroxyl-rhododendrol by tyrosinase. Conversely, the IC_{50} value was increased by an approximately equivalent amount when the expression/activity of tyrosinase was suppressed, revealing that the conversion of rhododendrol to hydroxyl-rhododendrol catalyzed by tyrosinase activity at a certain threshold dominantly causes the cytotoxic effects on melanocytes. It is important to clarify the criteria that determine whether melanocytes are tolerant to or sensitive to rhododendrol. We clearly demonstrate in this study that there is a certain threshold of tyrosinase activity that allows melanocytes to be tolerant or not, and this might depend on the mechanism by which hydroxyl-rhododendrol impairs melanocyte viability. It has been reported that some phenolic/catecholic compounds induce contact/occupational vitiligo because of their preferential cytotoxicity to melanocytes, which results in the initiation of apoptosis characterized by the reorientation of phosphatidylserine in the plasma membrane, DNA fragmentation and membrane blebbing [46]. The observation of the membrane blebbing of melanocytes treated with rhododendrol led us to hypothesize the possible involvement of apoptosis in the rhododendrol-induced melanocyte cytotoxicity for further investigation.

It is also of importance to pursue other possible etiologies independent of tyrosinase activity for the white blotching of the skin caused by rhododendrol since one melanocyte line with higher tyrosinase activity that we tested was tolerant to rhododendrol. Additionally, we also demonstrated that melanocytes could be rescued from the hydroxyl-rhododendrol-induced cell damage when the expression of tyrosinase was silenced. These findings led us to consider other possible points of action apart from the rhododendrol hydroxylation catalyzed by tyrosinase. It has been suggested that Tyrp1 plays a role in mediating the action of phenol/catechol derivatives resulting in vitiligo [30,47,48]. An examination of the possible contribution of Tyrp1 to the induction of cytotoxicity in rhododendrol-treated melanocytes remains to be pursued. The contribution of oxidative stress to the rhododendrol-induced decrease of melanocyte viability may be considered to be negligible based on the fact that treatment with antioxidants did not change the cytotoxic action of rhododendrol on melanocytes. Further, the depigmentation caused by rhododendrol is considered to be categorized as a chemical leukoderma distinct from contact/occupational vitiligo, and thus other possible mechanisms need to be addressed based on the limited information.

Overall, our data strongly suggest that hydroxyl-rhododendrol catalyzed by tyrosinase causes cytotoxicity in rhododendrol-sensitive melanocytes and is possibly one of the reasons for the skin depigmentation caused by the application of rhododendrol-containing products. The suppressed conversion of rhododendrol to its hydroxyl-derivative by inhibiting tyrosinase activity remarkably reduced its cytotoxicity to melanocytes, indicating the impact of a metabolite from the original compound whose cytotoxicity has not been previously considered, on the induction of depigmentation. Our findings provide new insights for a fundamental understanding of the mechanism that underlies skin depigmentation due to the application of brightening/lightening products. These results will be advantageous for the development of brightening/lightening materials so that such depigmentation problems never occur again, especially in the use of cosmetics and/or quasi-drugs.

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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.

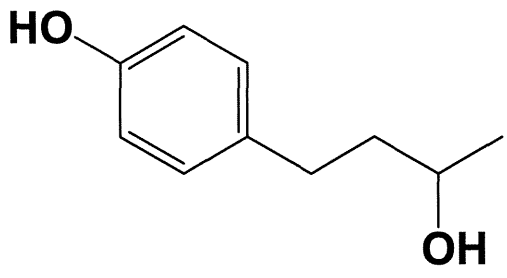

Fig. 1. Chemical structure of 4-(4-hydroxyphenyl)-2-butanol (HPB).

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.

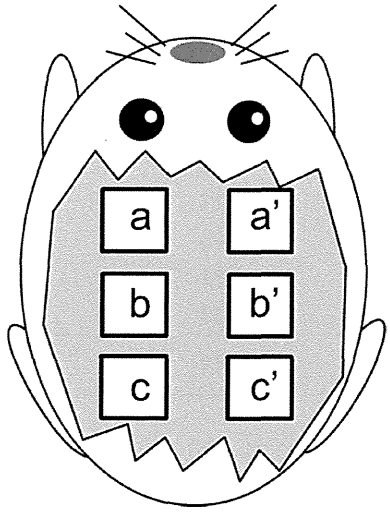

Fig. 2. Schematic representation for the fixed-dose experiment in brown guinea pigs. a: UV-treated and HPB-applied area (for biopsy), a': UV-treated and HPB-applied area (for recovery observation), b: no treatment area (for biopsy), b': no treatment area (for recovery observation), c: only UV-treated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

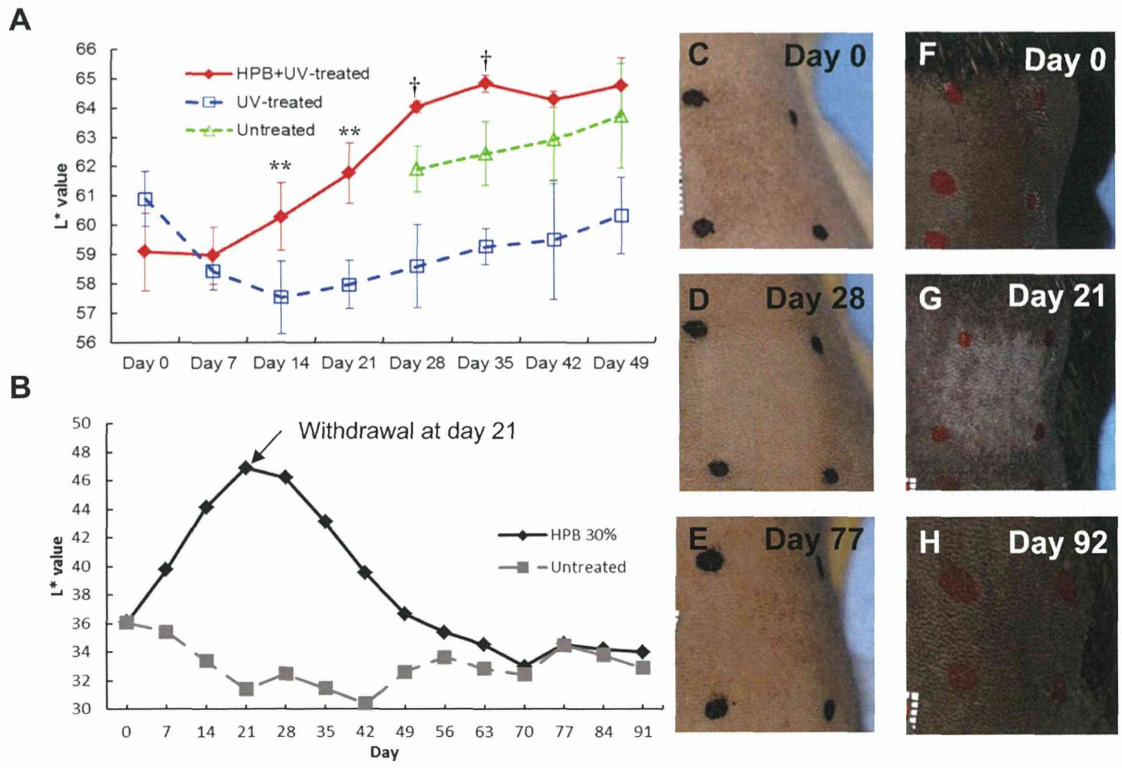

Fig. 3. Changes of skin brightness caused by HPB. (A) L*values of 3 dorsal areas in brown guinea pigs. Values are shown as means ± S.D. (Day 0-28, n = 5; Day 35, n = 4; Day 42-49, n = 3). (B) Time course of L*values for skin treated with 30% HPB and untreated skin of the black guinea pig. HPB was withdrawn 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 mm²
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

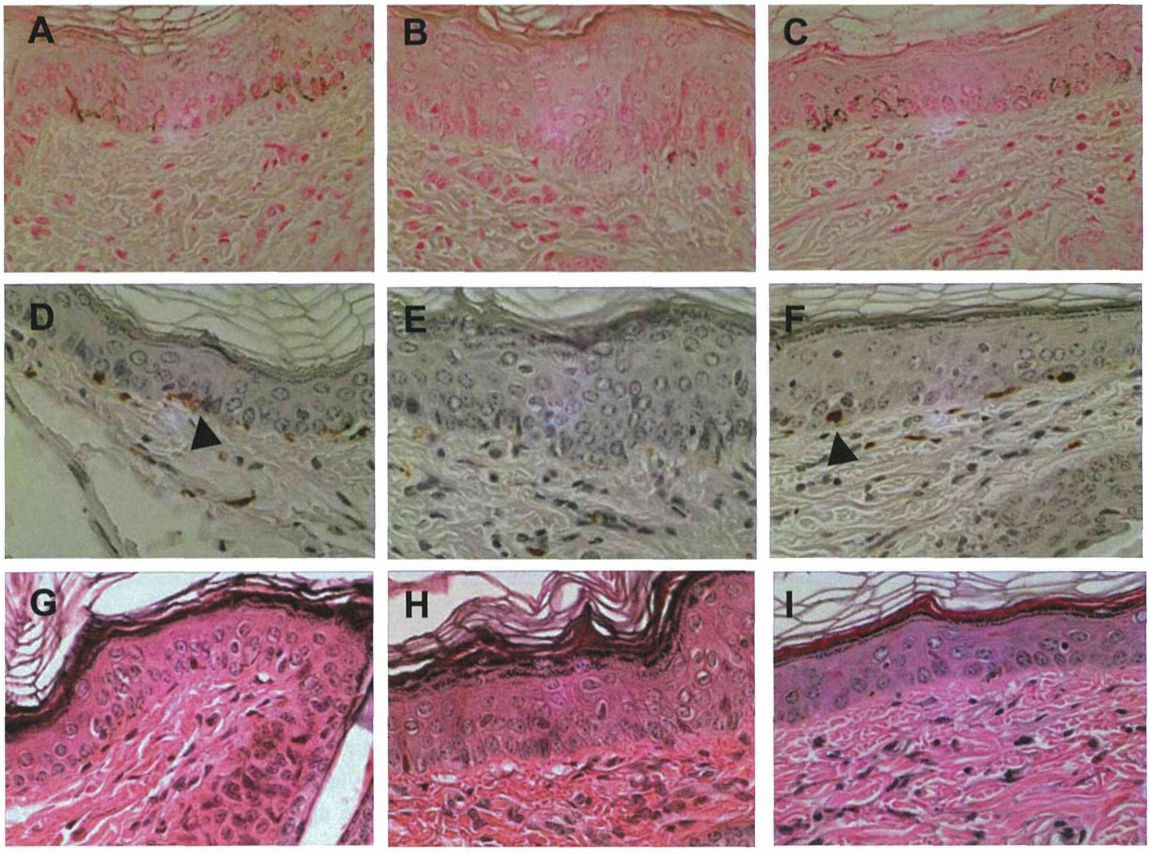

Fig. 4. Localization of melanocytes and melanin granules in the epidermis of brown guinea pigs. (A-C) Fontana-Masson silver stain, (D-F) immunohistochemical staining for S-100, (G-I) hematoxylin and eosin stains. Vertical paraffin sections were prepared from only UV-treated skin on day 41 (A, D, G), 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

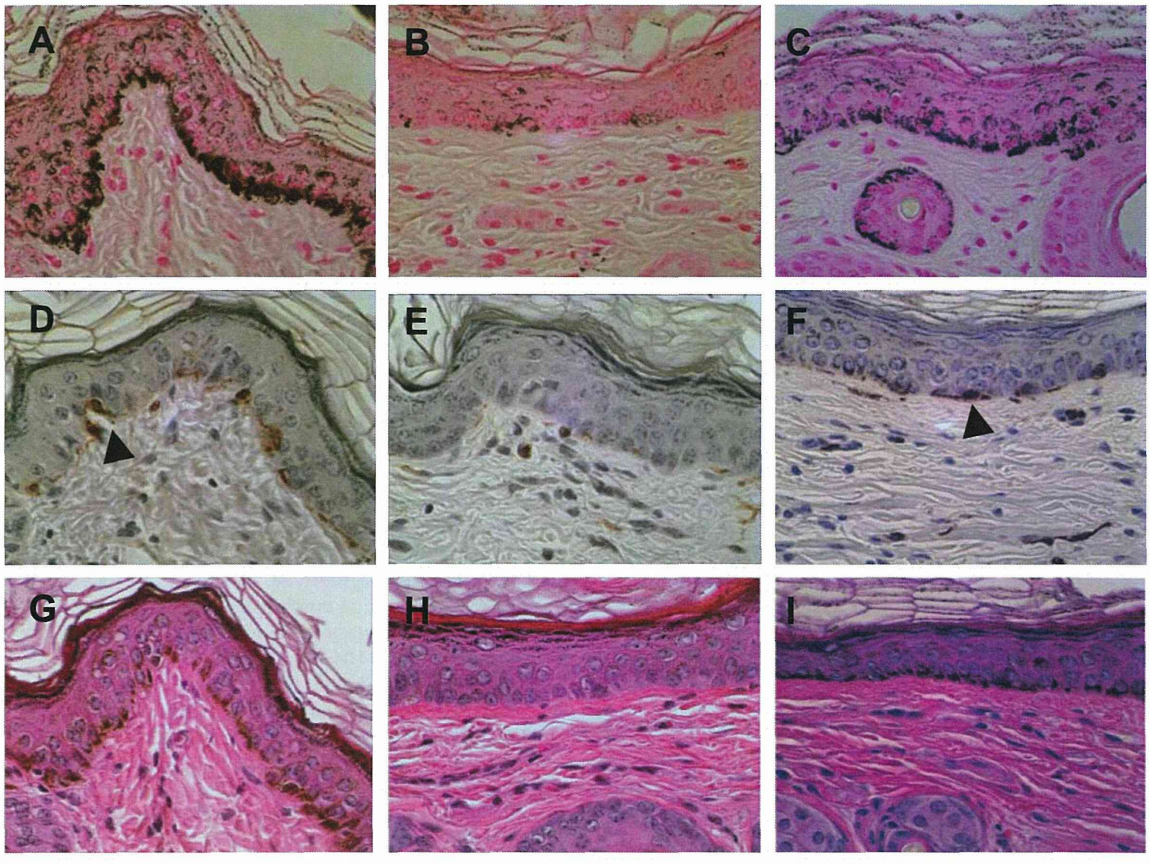

Fig. 5. Localization of melanocytes and melanin granules in the epidermis of a black guinea pig. (A-C) Fontana-Masson silver stain, (D-F) immunohistochemical staining for S-100, (G-I) hematoxylin and eosin stain. Vertical paraffin sections were prepared from untreated skin (A, D, G), from HPB-treated 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.

ACKNOWLEGMENT

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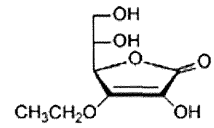
Key words: allergic contact dermatitis; cosmetics; 3-*o*-ethyl-L-ascorbic acid; skin-lightening agent; vitamin C ethyl.

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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Conflicts of interest: The authors declare no conflict of interests.

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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皮膚科学

皮膚のアレルギーのトピックス

Topics of immediate allergy in dermatology

矢上　晶子[1)]、松永　佳世子[2)]

要旨

皮膚のアレルギーは、食物アレルギー、花粉症、接触皮膚炎、遅延型アレルギー、アトピー性皮膚炎、薬疹など多岐にわたる。近年のトピックスとしては、石鹸に含まれた加水分解コムギ末による経皮・経粘膜感作により誘発された“小麦アレルギー”が挙げられる。本稿では、茶のしずく石鹸により誘発された即時型アレルギーの疫学調査を行ってきた、日本アレルギー学会「化粧品中のタンパク加水分解物の安全性に関する特別委員会（委員長：藤田保健衛生大皮膚科学教授　松永佳世子）」で集計された情報を基に本疾患について概説し、我々が日常的に使用している香粧品に潜むアレルギーのリスクについて述べる。

キーワード：即時型アレルギー、経皮感作、加水分解コムギ、小麦アレルギー、疫学調査

はじめに

皮膚のアレルギーは、食物アレルギー、花粉症、接触皮膚炎、遅延型アレルギー、アトピー性皮膚炎、薬疹など多岐にわたる。近年のトピックスとしては、“パンや麺類を食べたらアナフィラキシーショックになりました。その原因は石鹸でした”というフレーズに代表される、“石鹸に含まれた加水分解小麦末による経皮・経粘膜感作により誘発された小麦アレルギー”が挙げられる。これまでの即時型アレルギーのイメージは、“原因物質を接触した部位に誘発される接触蕁麻疹”や“経口的に摂取した食物や薬物による全身の蕁麻疹や呼吸困難”であった。しかし、この疾患は、原因は石鹸に含まれる加水分解コムギ末であるにも関わらず、多くの症例が小麦摂取後に突然眼瞼が腫脹したりアナフィラキシーが誘発されることを経験し、当初は誰も石鹸が原因であるとは気が付いていなかった点が従来の香粧品成分のアレルギーや食物アレルギーと大きく異なる点であった。現在、この（旧）茶のしずく石鹸により誘発された即時型アレルギーは全国で裁判が起こり、社会問題のひとつとなっている。

本稿では、茶のしずく石鹸により誘発された即時型アレルギーの疫学調査を行ってきた、日本アレルギー学会「化粧品中のタンパク加水分解物の安全性に関する特別委員会（委員長：藤田保健衛生大皮膚科学教授　松永佳世子）」で集計された情報を基に本疾患について概説し、我々が日常的に使用している香粧品に潜むアレルギーのリスクについて述べたい。

なお、本稿における内容は、日本アレルギー学会の以下のウェブサイトに掲載されており、これらの情報は毎月更新されているのでご参照されたい（2014年4月時点）http://www.jsaweb.jp/moduled/news_topics/index

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皮　膚　科　学

“(旧) 茶のしずく石鹸” とは

問題となった加水分解コムギ末である“グルパール19S”は2004年３月から2010年９月26日までに製造された“(旧) 茶のしずく石鹸（以下、茶のしずく石鹸と統一)”に含有されており、このグルパール19Sを含有した洗顔石鹸は 4650万８千個が延べ466万７千人に販売されたということが明らかにされている。これらの販売個数などの情報は本製品の販売が登録制であったため把握することが可能であった。この人数から予想すると、日本人女性の約10人に１人がこの石鹸を使用したということになる。

日本アレルギー学会「化粧品中のタンパク加水分解物の安全性に関する特別委員会」への登録症例数

2012年３月より調査を開始し、2014年４月20日時点で診断基準（表１）を満たす確実例は2,163例であった。性別では、女性2,074例（95.9％)、男性　89例（4.1%）だった。年齢は１歳（男児）から93歳（女性)、平均　45.8歳で、多くは20代から60代の女性だった。また、登録患者の都道府県別陽性症例数は、福岡県がトップで317例、次いで東京都127例、大阪府・北海道125例、第５位は広島県110例だった。つまり、本石鹸の使用者は圧倒的に女性であり、美容的な機能性を石鹸に求める世代が多く使用していた。また、各県別の登録症例数と石鹸の販売個数はある程度相関していた。

特徴的な臨床症状は

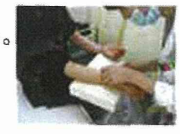
小麦摂取後の著しい眼瞼腫脹と重篤な即時型アレルギー反応であるアナフィラキシー症状が特徴的な臨床症状であった（図１)。本疾患は従来の小麦依存性運動誘発性アナフィラキシー

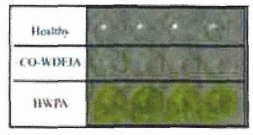
表１　茶のしずく石鹸等に含まれた加水分解コムギ（グルパール19S）による即時型小麦アレルギーの診断基準

(化粧品中のタンパク加水分解物の安全性に関する特別委員会作成 2011.10.11)

【確実例】
以下の1，2，3をすべて満たす。

1．加水分解コムギ(グルパール19S)を含有する茶のしずく石鹸等を使用したことがある。
2．以下のうち少なくとも一つの臨床症状があった。
2-1)加水分解コムギ(グルパール19S)を含有する茶のしずく石鹸等を使用して数分後から30分以内に，痒み，眼瞼浮腫，鼻汁，膨疹などが出現した。
2-2)小麦製品摂取後4時間以内に痒み，膨疹，眼瞼浮腫，鼻汁，呼吸困難，悪心，嘔吐，腹痛，下痢，血圧低下などの全身症状がでた。
3.以下の検査で少なくとも一つ陽性を示す(備考参照)。
3-1)グルパール19S 0.1%溶液，あるいは，それより薄い溶液でプリックテストが陽性を示す。
3-2)ドットブロット，ELISA，ウエスタンブロットなどの免疫学的方法により、血液中にグルパール19Sに対する特異的IgE抗体が存在することを証明できる。
3-3)グルパール19Sを抗原とした好塩基球活性化試験が陽性である。





【否定できる基準】
4.グルパール19S 0.1%溶液でプリックテスト陰性【疑い例】

1，2を満たすが3を満たさない場合は疑い例となる。
＊ただし1，2を満たすが3を満たさない場合でも，血液特異的IgE抗体価検査やプリックテストでコムギまたはグルテンに対する感作が証明され，かつω5グリアジンに対する過敏性がないか、コムギおよびグルテンに対する過敏症よりも低い場合は強く疑われる例としてよい。

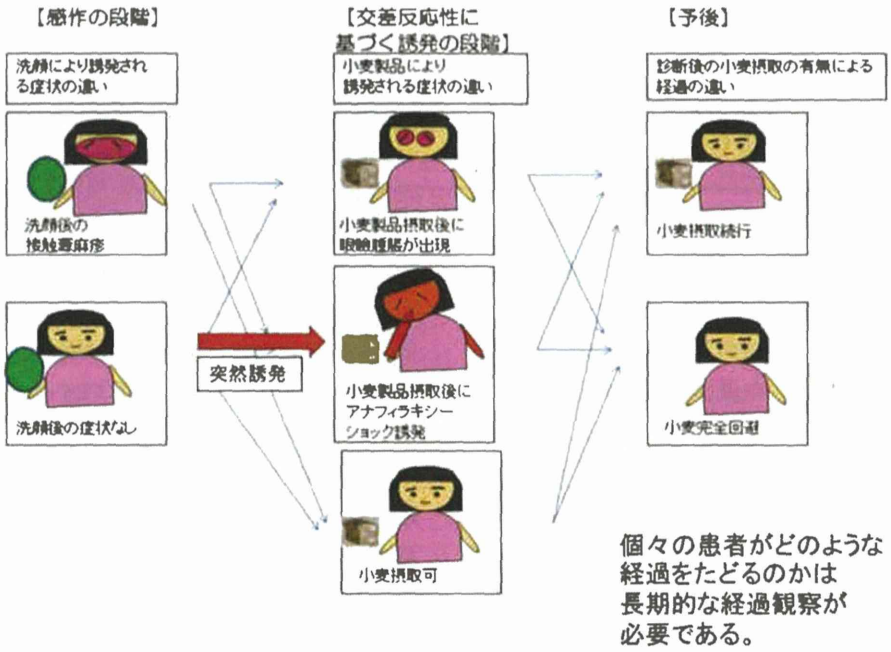


図１　「茶のしずく」石鹸使用者の接触蕁麻疹・小麦アレルギーの臨床症状は多彩である。

（FDEIA）と類似する点が多いが、小麦摂取後に軽い運動もしくは運動をしなくても症状が誘発される点がFDEIAと大きく異なる点であった。

洗顔後と小麦摂取後にそれぞれ症状が誘発された症例がある一方、洗顔時の症状はなく小麦摂取後のみ症状が誘発された症例もあり、その臨床症状は多彩であった。

洗顔後に眼瞼が腫れる、顔に蕁麻疹がでるなど、石鹸の接触部位の症状と小麦摂取後のアレルギー症状の両方の症状があった症例は67%、洗顔後の症状はなく小麦摂取後にアレルギー症状ありは30%で、97%の症例は小麦摂取後にアレルギー症状を発症していた。小麦摂取後の症状として、ほぼ全例に著明な眼瞼腫脹を認めていた（図２）。同時に誘発される全身症状として、アナフィラキシーショックは25%、ショック症状はないが呼吸困難・嘔吐や下痢を生じた症例は27%あり、合計で52%がアナフィラキシー症状を起こしていた。アナフィラキシー以外の蕁麻疹・眼の腫れ・鼻閉・鼻水・痒みなどは45%でみられた。症例の

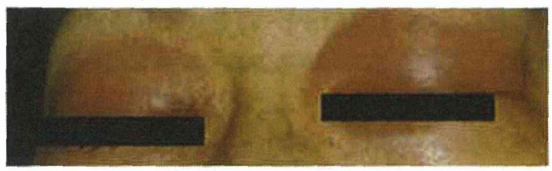


図２　特徴的な眼瞼の腫脹

中には洗顔後の症状がなかったため、小麦摂取後のアナフィラキシー症状を繰り返し、複数回救命センターへ搬送されていた症例もあった。一方、６割程度の症例は洗顔時に眼瞼の腫脹、蕁麻疹、痒みが誘発されていたが呼吸困難、ショック症状をきたした症例はなかった。つまり、経皮的に接触するのみでは重篤なアナフィラキシー症状は誘発されず、小麦製品として経口的に摂取しないと全身的な症状は誘発されるに至らないことが示された。

何が原因だったのか

近年、ヘアケア製品や基礎化粧品などに“加水

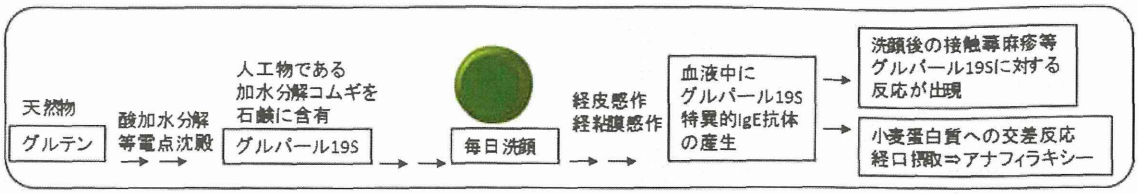


図３　本疾患の発症機序

分解コムギ”や“加水分解コラーゲン”など多くの“加水分解物”が含有されている。“加水分解物”とは、小麦などの天然の蛋白質原料を酵素や酸、アルカリで分解・加工したもので、これらの処理を施すことにより保湿性や使用感が高くなることから、多くの化粧水や石鹸などに含まれている。

本疾患の原因物質は、（旧）茶のしずく石鹸に入っていた加水分解コムギであるグルパール19S（片山化学工業研究所）であることが明らかにされている。これは天然のグルテンから酸や加熱を加え、加水分解物として人工的に作り出された物質である（図３）。石鹸使用者が毎日洗顔などで使用したことにより経皮感作が成立したとされている。

石鹸を頻回に、もしくは長期的に使用したから発症したのか

本石鹸は、2004年３月から2010年９月26日までに製造販売されたが、疫学調査で確実例として登録された症例の石鹸使用開始年を調査したところ、2004年は３例だったが、2005年に22例、2006年29例、2007年34例と徐々に増加し、2008年64例、2009年60例とピークになり、2010年33例、2011年１例となっていた。また、症例の多くは2010年および2011年に石鹸の使用を中止しており、これらは厚生労働省　医薬食品局安全対策課からの「コムギ加水分解物を含有する医薬部外品・化粧品による全身性アレルギーの発症について（2010年10月15日）」、および「コムギ加水分解物含有石鹸「茶のしずく石鹸」の自主回収について（2011年５月20日）」が公開された時期に一致していた。これらの石鹸使用者のアレルギーを発症した時期を確認すると、2005年に１例、2006年に６例、2007年に８例、2008年に36例、2009年に52例、2010年には73例が発症していた。しかしながら、厚労省の通達後の2011年に発症した症例も58例、2012年にも２例の登録があり、本事例のようにアウトブレークした疾患については、厚労省のみならず、医師および医療機関がより積極的に啓発活動を進める必要があったと認識した。

１人当たり使用した石鹸の数では、10個が最も多く23例、20個が22例だった。最少１個、最多70個で平均は15.6個だった。やはり多くの症例は当該石鹸を複数使用した後に発症していたが、１個の使用でも発症した症例があることは注目すべきと考える。また、１日の使用回数は、１日１回は74例、２回114例、３回13例、４回４例で平均1.7回であった。石鹸使用者のほとんどは洗顔に使用していたが、顔面だけの使用は64％、顔と体は17％、顔と首は２％、顔・腕・手は１％だった。

症例供覧

以下に、典型的な症例を供覧する。

症例　47歳女性

主訴　食後のアナフィラキシー

既往歴　花粉症

現病歴　約３年間、茶のしずく石鹸を使用した。原因不明の食事摂取後のアナフィラキシー症状のため過去１年間に４回救命センターに搬送されている。そのうち２回は、雪かきの後に誘発されて

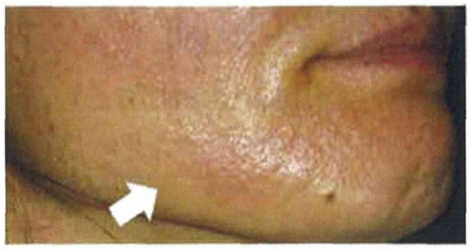


図４　洗顔使用後の顔面症状（痒みを伴う発赤）

いる。その後、洗顔石鹸使用後に顔面の痒みが出現するようになった（図４）。

各種検査結果（2010年夏）：

血清学的検査：非特異IgE抗体318IU/ml、コムギ特異IgE抗体値 2.41（クラス２）UA/ml、グルテン特異IgE抗体値 4.05（クラス３）UA/ml、ω-グリアジン特異IgE抗体値陰性、グルパール19S特異IgE115.5Unit

皮膚テスト（プリックテスト）：コムギ陽性、グルパール19S陽性

確定診断：臨床経過および検査結果より、本疾患と確定診断した。

経過：患者は現在（2014年４月）も小麦の摂取を制限し生活している。2014 年２月の時点での検査結果は、非特異IgE抗体307IU/ml、コムギ特異IgE抗体値 0.59（クラス１）UA/ml、グルテン特異IgE抗体値 0.67（クラス１）UA/ml、ω-グリアジン特異IgE抗体値陰性、グルパール19S特異IgE31.0Unitである。小麦の摂取を避ければ症状は誘発されずに生活できているが、今後は小麦の摂取を希望している。

グルパール19S以外の加水分解コムギを含有する香粧品は安全か？

グルパール19Sが含有された茶のしずく石鹸以外の製品（石鹸等）でも同様の小麦アレルギー症例もあるようだが、グルパール19S以外の加水分解コムギを含有した製品で小麦アレルギーが複数の症例に発症したという報告はない。よって、加水分解コムギを含むその他の製品までもが危険であると断定することはできないが、同じようなことが他の製品でも起こる可能性も否定できない。小麦アレルギーと診断された症例については、その他の加水分解コムギ含有製品の使用についても控えるよう指導している。

さいごに

本稿では、“皮膚のアレルギーのトピックス”として、加水分解コムギ末により経粘膜・経皮的に感作された小麦アレルギーを述べた。現在、化粧品や医薬部外品（薬用化粧品）における製造販売前の化粧品成分の即時型アレルギー試験は必須項目には入っておらず、安全性を確保する市販前の試験法の標準化と市販後において化粧品による有害事象を早期に把握するシステムの構築は急務といえる。今後、社会的な取り組みが進み、化粧品の安全性が確保されることを期待したい。

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