Method. The test drugs were applied to one site on both upper arms and to two sites on both forearms (total of six application sites) twice daily (morning and after bathing in the evening) for 3 weeks.

Evaluation. Subjects were asked to rate objective symptoms (scaling, erythema, papules and edema) and subjective symptoms (soreness, heat and itchiness) on a scale of 0–3 (3, severe; 2, moderate; 1, mild; and 0, none). They were also asked to record the presence of any other symptoms. When the symptom score was either 1 or 2, they were required to send pictures of the cutaneous symptoms to the observer and they were provided instructions regarding continuation of the test and treatment. We instructed the participants to stop the test prior to assessment by the observer if the symptom score was more than 2 or if they suffered another adverse event. In addition, the observer or test administrator inspected the participant's arm when the test was finished.

Statistical analysis

The irritation scores between study 1 and study 2 at 2 and 24 h after patch removal, and the percentage of objective symptoms between study 3 and study 4 were compared using Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Reproducibility of PT

The distribution of the irritation scores for test drugs and control substances in study 1 and study 2 is shown in Figure 1. The irritation scores of each test drug at 2 (Fig. 1a) or 24 h (Fig. 1b) after patch removal in both studies were similar (Fig. 1a, $y = 0.9011x - 0.0991$, $r = 0.8714$, $P < 0.01$; Fig. 1b, $y = 1.2223x + 0.0388$, $r = 0.9222$, $P < 0.01$). Moreover, the mean scores were similar regardless of the duration of drug exposure.

The mean irritation scores ranged 0.03–0.43 for the negative control drugs and 0.07–2.07 for positive controls; a clear difference between the negative and positive controls in the range of values was observed (Fig. 1c, $r = 0.7429$; Fig. 1d, $r = 0.6514$). The mean irritation scores of 0.2% SLS solution in study 1 and study 2 were 0.77 and 0.93 at 2 h after patch removal, and 1.14 and 1.60 at 24 h after patch removal, respectively. The mean irritation scores of 0.1% SLS solution in study 1 and study 2 were 0.50 and 0.52 at 2 h after patch removal, and 0.59 and 0.73 at 24 h after patch removal, respectively. The results of the SLS PT suggest that the skin irritation scores are concentration-dependent.

Use test with twice daily application to the arm for 3 weeks

Table 3 show the mean skin irritation scores 24 h after drug patch removal, the number of individuals who developed skin symptoms or abnormal sensation, and the nature of the skin manifestations in study 3 and study 4. The distribution of the percentage of subjects positive for symptoms provoked by the test drugs in study 3 and study 4 is shown in Figure 2 (Fig. 2a, $r = 0.8414$, $P < 0.05$; Fig. 2b, $r = 0.7651$; Fig. 2c, $r = 0.8510$, $P < 0.05$). The percentage of positive symptoms was similar between the two groups.

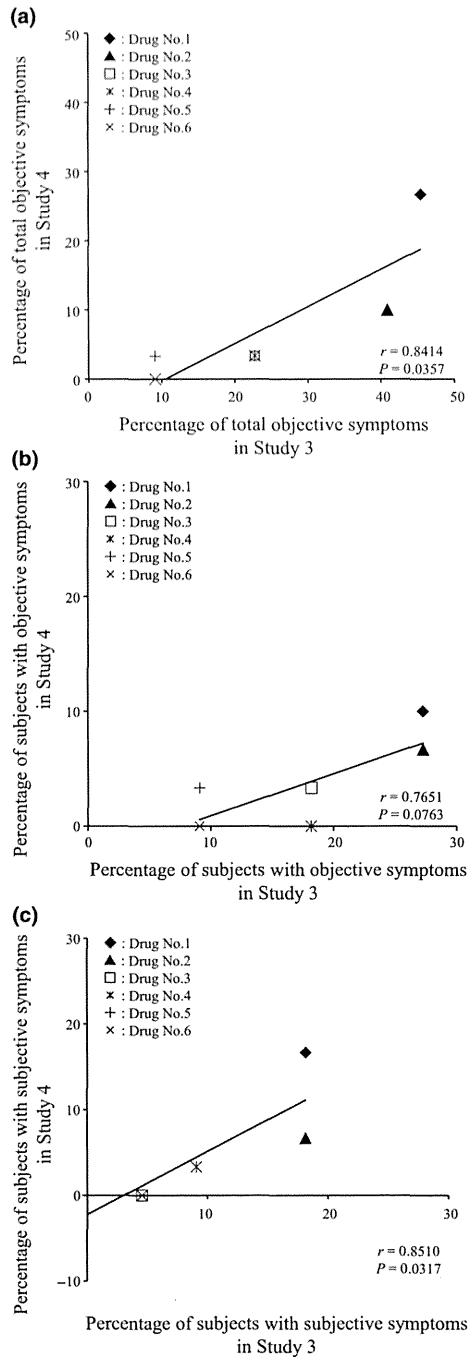

Figure 2. Correlation between the number of subjects and symptoms in study 3 and study 4. (a) Percentage of total objective symptoms. (b) Percentage of subjects with objective symptoms. (c) Percentage of subjects with subjective symptoms.

With respect to the nature of the symptoms, redness was observed most often, which was described as transient (disappearing within 0.5–1 h) and associated with an elevation of body temperature due to exercise, drinking and high ambient temperature. Drug no. 1 and no. 2 resulted in skin peeling-like features, which might have been due to hardening of the topical drug.

During the application test, one subject was suspected to have an allergic reaction to test drugs no. 1 and 2, and the application of these drugs was discontinued on day 2. Another

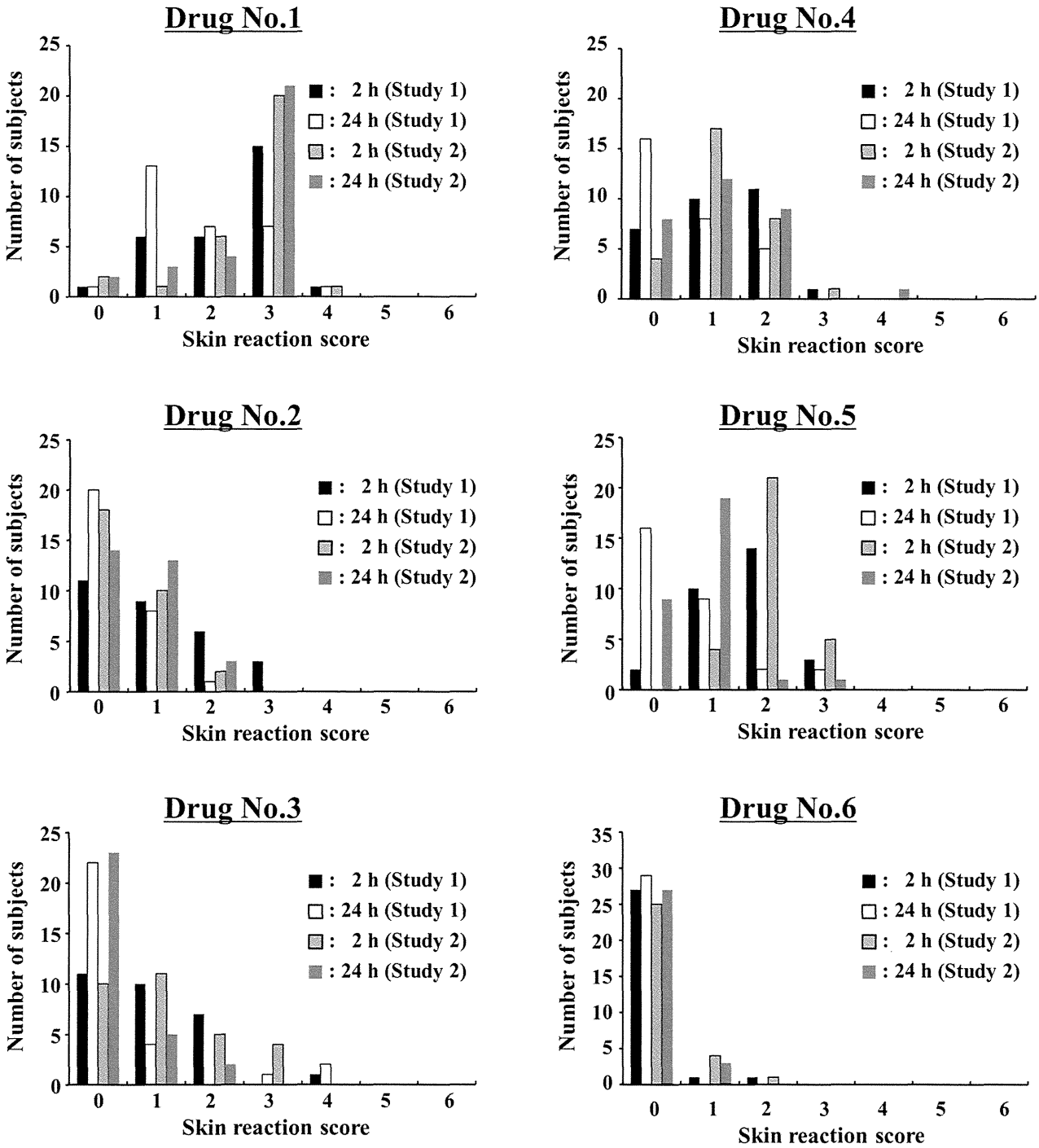

Figure 3. Patch testing irritation scores at 2 and 24 h after patch removal for each test drug in study 1 and study 2.

subject complained of itchiness on day 12 of application of test drug no. 1, and it was subsequently discontinued on day 14 because it was difficult to continue the test (study 3). One subject had a papule on day 8 of application of test drugs no. 1 and no. 2, and they were discontinued on day 9. Another subject had a papule on day 6 of application of test drugs no. 1, no. 2 and no. 3, and they were discontinued on day 7 (study 4).

Relationship between the results of PT and the use test with twice daily application for 3 weeks

Figure 3 shows the PT results of six topical drugs. Specifically, the skin reaction scores 2 and 24 h after patch removal are shown. Over half of the subjects had a score of 3 after exposure to drug no. 1 (mean irritation score 24 h after patch removal, ≥1.5). Most subjects had a score of 0 after exposure to drug no. 6 (mean irritation score 24 h after patch removal, <0.2). The scores of drugs no. 2–5 (mean irritation scores 24 h after patch removal, 0.2–1.4) varied from 0 to 4. When the test drugs were classified according to their skin irritation scores, it was found that the number of subjects with skin symptoms increased as the mean score rose, suggesting that the skin irritation scores correlate with skin symptoms (Fig. 4).

Table 4 shows the use test results including the number of subjects with objective or subjective symptoms, reaction score and assessment periods. Objective or subjective symptoms were documented from days 1–21. The total symptom scores of each drug, excluding transient redness, increased as the mean score rose.

DISCUSSION

Patch testing has been used to evaluate the skin irritancy of cosmetics, quasi-drugs and topical drugs. However, detailed evaluation of the reproducibility of this method or the significance of its results in relation to skin irritation on clinical application of the drug has not been examined.[23–25]

In this study, participants were selected at random who had normal back skin and on inspection. Biological assessments of barrier function such as transepidermal water loss, skin hydration and pH were not performed, and menstrual cycle was not considered in the selection criteria. PT of 55 topical drugs and seven control substances was performed with either a 24- or 48-h patch application (study 1 and 2, respectively) on two groups of subjects in January (study 1) and in June (study 2). The mean skin irritation scores[2–5] were similar in both studies, suggesting that the PT skin irritation score is reliable, even for a group of approximately 30 individuals with different genetic backgrounds and living environments.

Furthermore, the skin irritation score of the positive and negative control drugs were clearly different, and PT using different concentrations of SLS solutions revealed that the skin irritation score was concentration-dependent. As the skin reaction after 24 h of exposure was similar to that after 48 h of exposure, the 24-h closed test is considered sufficient for testing skin irritancy, and is also more convenient for the patient and examiner.[5] The above findings suggest that PT can identify skin irritancy of topical drugs and that PT results reflect the intensity of irritant reactions. Thus, this study confirms the reliability of PT.

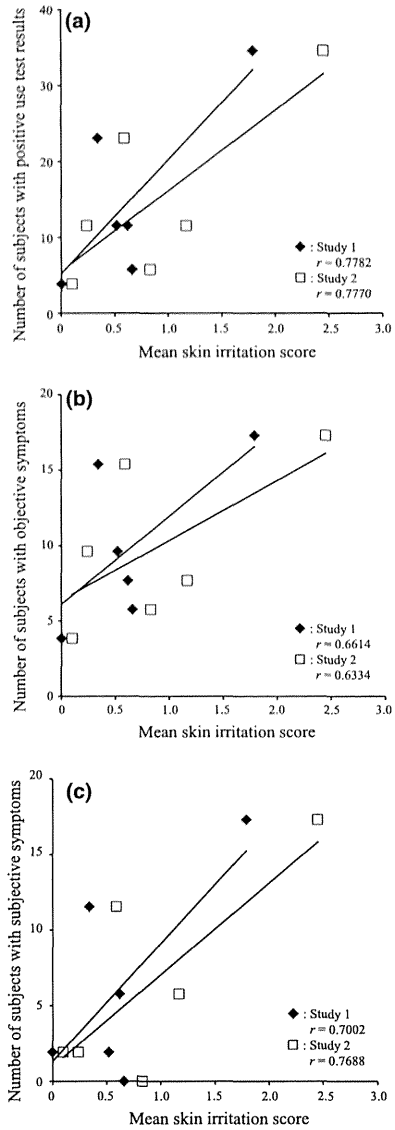

Figure 4. Relationship between patch testing-derived mean skin irritation scores at 24 h after patch removal and repeated open application test results. (a) Number of subjects with positive use test results. (b) Number of subjects with objective symptoms. (c) Number of subjects with subjective symptoms.

It is important that the cutaneous safety of topical drugs is tested on human subjects, and it is desirable to use the repeat open application test that reflects the clinical use of the drug. In this study, six topical drugs with different irritation scores were applied to the arms of two groups of 22–30 subjects, and symptoms were documented from days 1–21. It was found that the number of positive symptoms was similar between the

Table 4. Use test results: number of subjects with objective and subjective reactions and their scores

		Scaling			Erythema			Redness (transient)				Papule			Soreness			Heat			Itchiness		
Drug no.	Assessment period	0	1	2	0	1	2	0	1	2	3	0	1	2	0	1	2	0	1	2	0	1	2
1	Days 1–7		–		51	0	1	47	5	0	0	49	2	1	49	0	3		–		52	0	0
	Days 8–14		–		50	2	0	52	0	0	0	51	1	0	52	0	0		–		49	0	3
	Days 15–21		–		49	3	0	52	0	0	0	52	0	0	52	0	0		–		52	0	0
	Total score		–			7		5					5			6			–			6	
2	Days 1–7		–		51	0	1	45	6	1	0	49	2	1	49	2	1		–			–	
	Days 8–14		–		52	0	0	50	1	1	0	50	1	1	51	1	0		–			–	
	Days 15–21		–		52	0	0	49	3	0	0	52	0	0	52	0	0		–			–	
	Total score		–			2		16					7			5			–			–	
3	Days 1–7	52	0	0		–		45	6	1	0	50	2	0	51	1	0		–			–	
	Days 8–14	52	0	0		–		50	2	0	0	52	0	0	52	0	0		–			–	
	Days 15–21	50	2	0		–		49	3	0	0	52	0	0	52	0	0		–			–	
	Total score		2			–		13					2			1			–			–	
4	Days 1–7		–		52	0	0	48	3	1	0		–			–		52	0	0		–	
	Days 8–14		–		52	0	0	47	1	4	0		–			–		51	0	1		–	
	Days 15–21		–		50	2	0	51	1	0	0		–			–		51	1	0		–	
	Total score		–			2		15					–			–			3			–	
5	Days 1–7	52	0	0	51	1	0	47	3	2	0		–			–			–			–	
	Days 8–14	52	0	0	52	0	0	49	2	0	1		–			–			–			–	
	Days 15–21	51	1	0	51	1	0	50	1	0	1		–			–			–			–	
	Total score		1			2		14					–			–			–			–	
6	Days 1–7		–		51	1	0	48	3	1			–		51	1	0		–			–	
	Days 8–14		–		52	0	0	50	1	1			–		52	0	0		–			–	
	Days 15–21		–		52	0	0	51	1	0			–		52	0	0		–			–	
	Total score		–			1		9					–			1			–			–	

two groups, and the number of subjects with skin symptoms increased as the mean score rose. Furthermore, total symptom scores, excluding transient redness, increased as the mean skin irritation scores rose.

Skin irritation was observed despite the application of anti-inflammatory drugs including steroid and those with anti-inflammatory ingredients in PT and use test. Therefore, PT and use test can detect skin irritation without being affected by anti-inflammatory effects. Application of drugs including steroid resulted in a bluish tinge to the skin at 2 h after chamber removal in PT. Compared with the PT skin irritation scores 2 h after patch removal, the scores at 24 h after patch removal showed a higher correlation with the number of subjects who developed skin symptoms in the use test (data not shown). It has been reported that skin irritant reactions were strongest at 24 h after patch removal and tended to be weaker at 48 h after patch removal.[5] These observations suggest that 24 h after patch removal is an optimal time point for reading the results of PT for potential skin irritants. Drug no. 1 had a high mean skin irritation score on PT (scores, >3), a higher number of discontinued subjects and was associated with skin symptoms, while drug no. 6 had a low PT score associated with few skin symptoms. Other drugs were associated with skin symptoms and had to be discontinued in several subjects, possibly caused by PT reactions with scores of 3. Further study is needed to clarify the significance of PT and skin irritation scores in determining skin safety.

The transient redness appeared when the body temperature rose and disappeared after 0.5–1 h, and was not perceived by patients as irritative. Presumably, it was caused by transient vasodilation without inflammatory cell infiltration.[28,29] The total score of objective symptoms excluding transient redness also increased with the skin irritation mean score, suggesting that the skin irritation scores correlate with skin symptoms (Table 4).

Drugs no. 1 (gel) and no. 2 (lotion) resulted in skin peeling-like features but no erythema or itchiness were observed (Table 3). This was thought to be due to hardening of the topical drugs rather than inflammation. The above-mentioned drugs, those with high PT scores or use test scores, those that provoked symptoms, and those that induced skin peeling-like symptoms require further study to determine whether these are related to skin irritation.

The above-mentioned observations suggest that PT is useful in predicting the safety of topical drugs in addition to the repeated open application test that simulates the clinical use of the test drug. In this study, the participants had normal skin on inspection; when we study the relationship between normal skin and sensitive skin, we should examine biological assessment of barrier function. As transient redness was observed in the repeated open application test in this study, the erythema in PT is thought to reflect vasodilation combined with an irritant reaction with an inflammatory cell infiltrate, especially in commercially available drugs containing active ingredients.

In conclusion, the mean skin irritation score derived from PT is a reproducible system for evaluating the safety of topical drugs and accurately reflects the severity of skin irritation. Also, the skin irritation score of PT closely correlated with the number of skin symptoms observed in the repeated open application test that simulated the clinical use of the drug. PT is a useful method for predicting the safety of topical drugs.

CONFLICT OF INTEREST: The authors have no conflict of interest.

REFERENCES

1 Society of Regulatory Science of Medical Products. Drug Approval and Licensing Procedure, 2010.
2 Kawamura T, Shoji S, Masuda T *et al.* Basic studies on the standardization of patch test. *Jpn J Dermatol* 1970; **80**: 301–314 (in Japanese).
3 Sugai T. Contact dermatitis and patch test. *Skin Res* 1977; **19**: 210–222.
4 Sugai T. Delayed irritation ratio in prophetic patch tests. *Skin Res* 1985; **27**: 793–803.
5 Kanto H, Washizaki K, Ito M *et al.* Optimal patch application time in the evaluation of skin irritation. *J Dermatol* 2013; **40**: 363–369.
6 Basketter DA, Reynolds F, Rowson M, Talbot C, Whittle E. Visual assessment of human skin irritation: a sensitive and reproducible tool. *Contact Dermatitis* 1997; **37**: 218–220.
7 Mehling A, Chkarnat C, Degwert J *et al.* Interlaboratory studies with a proposed patch test design to evaluate the irritation potential of surfactants. *Contact Dermatitis* 2010; **62**: 157–164.
8 Basketter DA, Griffiths HA. A study of the relationship between susceptibility to skin stinging and skin irritation. *Contact Dermatitis* 1993; **29**: 185–188.
9 Basketter DA, Chamberlain M, Griffiths HA, Rowson M, Whittle E, York M. The classification of skin irritants by human patch test. *Food Chem Toxicol* 1997; **35**: 845–852.
10 York M, Basketter DA, Cuthbert JA, Neilson L. Skin irritation testing in man for hazard assessment–evaluation of four patch systems. *Hum Exp Toxicol* 1995; **14**: 729–734.
11 Tupker RA, Willis C, Berardesca E *et al.* Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1997; **37**: 53–69.
12 Foy V, Weinkauf R, Whittle E, Basketter DA. Ethnic variation in the skin irritation response. *Contact Dermatitis* 2001; **45**: 346–349.
13 Basketter DA, Miettinen J, Lahti A. Acute irritant reactivity to sodium lauryl sulfate in atopics and non-atopics. *Contact Dermatitis* 1998; **38**: 253–257.
14 Basketter DA, York M, McFadden JP, Robinson MK. Determination of skin irritation potential in the human 4-h patch test. *Contact Dermatitis* 2004; **51**: 1–4.
15 Brasch J, Becker D, Effendy I. Reproducibility of irritant patch test reactions to sodium lauryl sulfate in a double-blind placebo-controlled randomized study using clinical scoring: results from a study group of the German Contact Dermatitis Research Group (Deutsche Kontaktallergie-Gruppe, DKG). *Contact Dermatitis* 1999; **41**: 150–155.
16 Basketter DA, Griffiths HA, Wang XM, Wilhelm KP, McFadden J. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. *Contact Dermatitis* 1996; **35**: 208–213.
17 Basketter DA, Scholes EW, Kimber I. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. *Food Chem Toxicol* 1994; **6**: 543–547.
18 Whittle E, Basketter DA. The *in vitro* skin corrosivity test. Development of method using human skin. *Toxicol In Vitro* 1993; **7**: 265–268.
19 Whittle E, Basketter DA. The *in vitro* skin corrosivity test. Comparison of *in vitro* human skin with *in vivo* data. *Toxicol In Vitro* 1993; **7**: 269–273.
20 Basketter DA, Selbie E, Scholes EW, Lees D, Kimber I, Botham PA. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. *Food Chem Toxicol* 1993; **31**: 63–67.
21 Botham PA, Basketter DA, Maurer T, Mueller D, Potokar M, Bontinck WJ. Skin sensitization–a critical review of predictive test methods in animals and man. *Food Chem Toxicol* 1991; **29**: 275–286.
22 Tornier C, Rosdy M, Maibach HI. *In vitro* skin irritation testing on reconstituted human epidermis: reproducibility for 50 chemicals tested with two protocols. *Toxicol In Vitro* 2006; **20**: 401–416.
23 Basketter D, Gilpin G, Kuhn M, Lawrence D, Reynolds F, Whittle E. Patch tests versus use tests in skin irritation risk assessment. *Contact Dermatitis* 1998; **39**: 252–256.
24 Roggeband R, Helmlinger G, Smith I, Wilhelm KP, Ryan CA, Gerberick GF. A skin sensitization safety assessment of a new bleach activator technology in detergent applications. *Contact Dermatitis* 2002; **46**: 211–219.
25 Zaghi D, Maibach HI. Quantitative relationships between patch test reactivity and use test reactivity: an overview. *Cutan Ocul Toxicol* 2008; **27**: 241–248.
26 Judge MR, Griffiths HA, Basketter DA, White IR, Rycroft RJ, McFadden JP. Variation in response of human skin to irritant challenge. *Contact Dermatitis* 1996; **34**: 115–117.
27 Kawai K. Proposal of a new standard for skin irritation reaction in patch testing. *Vis Dermatol* 2004; **3**: 74–81 (in Japanese).
28 Nickoloff BJ. Immunologic reactions triggered during irritant contact dermatitis. *Am J Contact Dermat* 1998; **9**: 107–110.
29 Tabata N, Tagami H, Kligman AM. A twenty-four-hour occlusive exposure to 1% sodium lauryl sulfate induces a unique histopathologic inflammatory response in the xerotic skin of atopic dermatitis patients. *Acta Derm Venereol* 1998; **78**: 244–247.

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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.

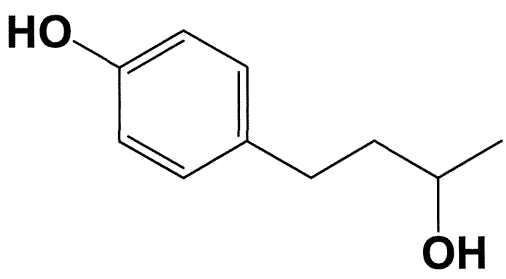

Fig. 1. Chemical structure of 4-(4-hydroxyphenyl)-2-butanol (HPB).

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.

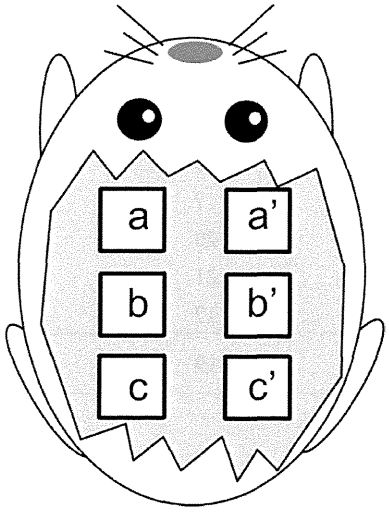

Fig. 2. Schematic representation for the fixed-dose experiment in brown guinea pigs. a: UV-treated and HPB-applied area (for biopsy), a': UV-treated and HPB-applied area (for recovery observation), b: no treatment area (for biopsy), b': no treatment area (for recovery observation), c: only UV-treated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

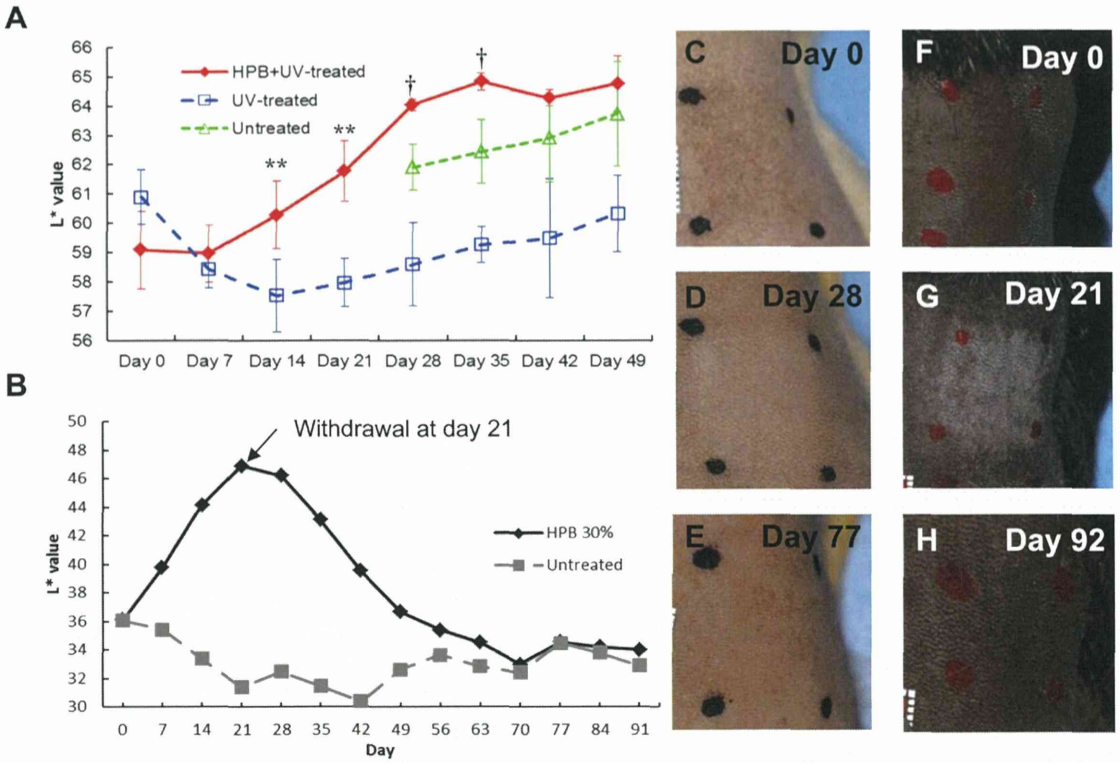

Fig. 3. Changes of skin brightness caused by HPB. (A) L*values of 3 dorsal areas in brown guinea pigs. Values are shown as means ± S.D. (Day 0-28, n = 5; Day 35, n = 4; Day 42-49, n = 3). (B) Time course of L*values for skin treated with 30% HPB and untreated skin of the black guinea pig. HPB was withdrawn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

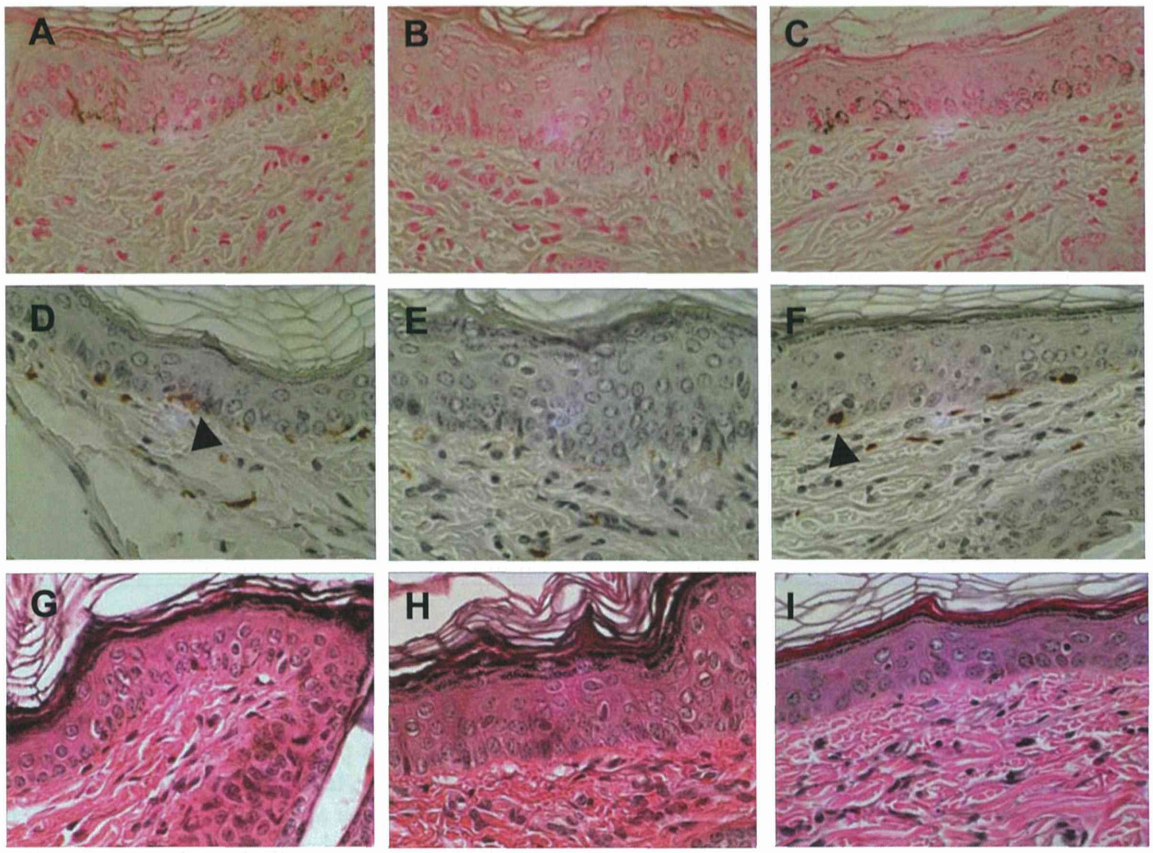

Fig. 4. Localization of melanocytes and melanin granules in the epidermis of brown guinea pigs. (A-C) Fontana-Masson silver stain, (D-F) immunohistochemical staining for S-100, (G-I) hematoxylin and eosin stains. Vertical paraffin sections were prepared from only UV-treated skin on day 41 (A, D, G),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

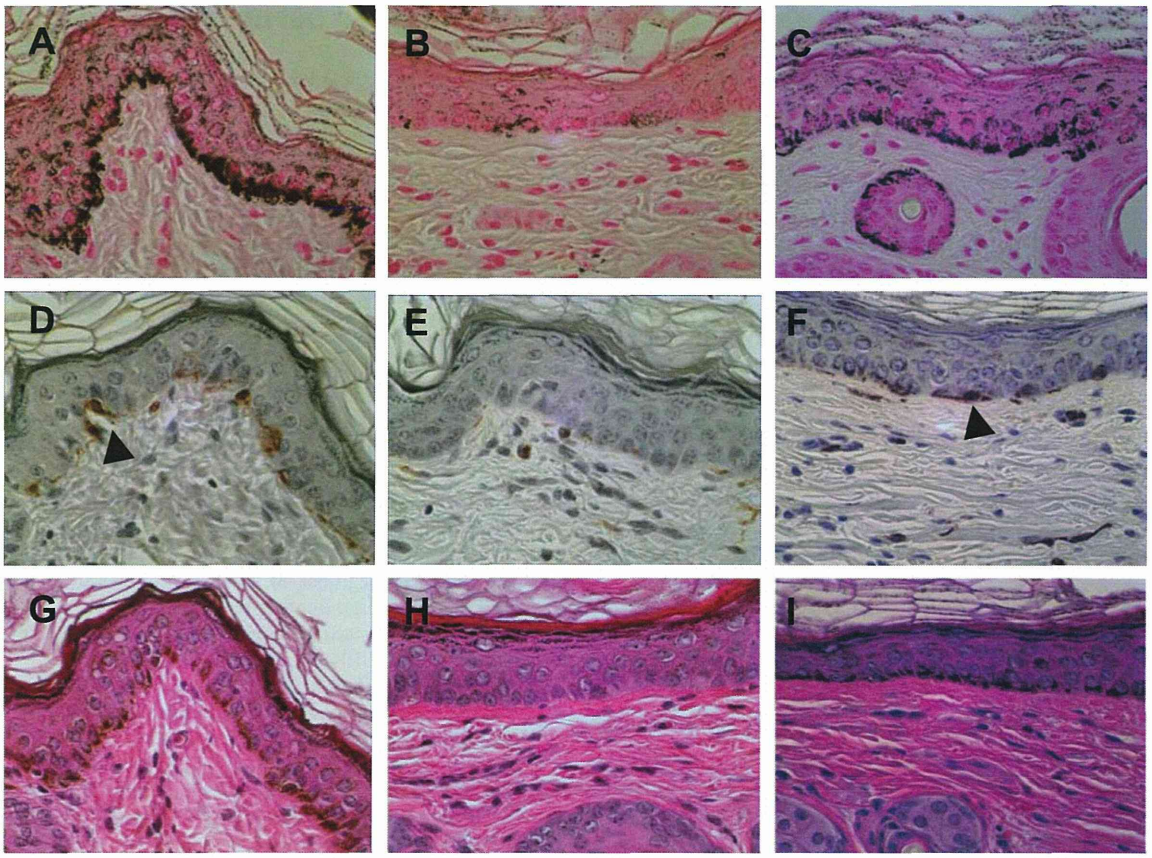

Fig. 5. Localization of melanocytes and melanin granules in the epidermis of a black guinea pig. (A-C) Fontana-Masson silver stain, (D-F) immunohistochemical staining for S-100, (G-I) hematoxylin and eosin stain. Vertical paraffin sections were prepared from untreated skin (A, D, G), from HPB-treated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

Arndt, K.A. and Fitzpatrick, T.B. (1965): Topical use of hydroquinone as a depigmenting agent. J. Amer. Med. Assoc., **194**, 965-967.

Bleehen, S.S., Pathak, M.A., Hori, Y. and Fitzpatrick, T.B. (1968): Depigmentation of skin with 4-isopropylcatechol, mercaptoamines, and other compounds. J. Invest. Dermatol., **50**, 103-117.

Boissy, R.E. and Manga, P. (2004): On the etiology of contact/occupational vitiligo. Pigment Cell Res., **17**, 208-214.

Carruthers, W. (1978): Modern methods of organic synthesis. (Carruthers, W. and Coldham, I.), pp.407-432., Cambridge University Press, Cambridge.

Chou, W.C., Takeo, M., Rabbani, P., Hu, H., Lee, W., Chung, Y.R., Carucci, J., Overbeek, P. and Ito, M. (2013): Direct migration of follicular melanocyte stem cells to the epidermis after wounding or UVB irradiation is dependent on Mc1r signaling. Nat. Med., **19**, 924-929.

Denton, C.R., Lerner, A.B. and Fitzpatrick, T.B. (1952): Inhibition of melanin formation by chemical agents. J. Invest. Dermatol., **18**, 119-135.

Ebner, V.H., Helletzgruber, M., Kolbe, H., Weissel, M. and Winker, N. (1979): Vitiligo durch p-tert. butylphenol, Beitrag zur frage interner manifestationen dieser berufserkrankung. Derm. Beruf Umwelt, **27**, 99-104.

Frenk, E. and Loi-Zedda, P. (1980): Occupational depigmentation due to a hydroquinone-containing photographic developers. Contact Dermatitis, **6**, 238-239.

Fukuda, Y., Nagano, M., Arimatsu, Y. and Futatsuka, M. (1998a): An experimental study on depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone in C57 black mice. J. Occup. Health, **40**, 97-102.

Fukuda, Y., Nagano, M. and Futatsuka, M. (1998b): Occupational leukoderma in workers engaged in 4-(p-hydroxyphenyl)-2-butanone manufacturing. J. Occup. Health, **40**, 118-122.

Fukuda, Y., Nagano, M., Tsukamoto, K. and Futatsuka, M. (1998c): *In vitro* studies on the depigmenting activity of 4-(p-hydroxyphenyl)-2-butanone. J. Occup. Health, **40**, 137-142.

Gebhart, W., Luger, T. and Niebauer, G. (1980): Vitiligo due to p-tert-butyl phenol. Ann. Dermatol. Venereol., **107**, 809-814.

Gellin, G.A., Maibach, H.I., Misiaszek, M.H. and Ring, M. (1979): Detection of environmental depigmenting substances. Contact Dermatitis, **5**, 201-213.

Hariharan, V., Klarquist, J., Reust, M.J., Koshoffer, A., McKee, M.D., Boissy, R.E. and Le Poole, I.C. (2010): Monobenzyl ether of hydroquinone and 4-tertiary butyl phenol activate markedly different physiological responses in melanocytes: relevance to skin depigmentation. J. Invest. Dermatol., **130**, 211-220.

Jimbow, K., Obata, H., Pathak, M.A. and Fitzpatrick, T.B. (1974): Mechanism of depigmentation by hydroquinone. J. Invest. Dermatol., **62**, 436-449.

Kahn, G. (1970): Depigmentation caused by phenolic detergent germicides. Arch. Dermatol., **102**, 177-187.

Kersey, P. and Stevenson, C.J. (1981): Vitiligo and occupational exposure to hydroquinone from servicing self-photographing machines. Contact Dermatitis, **7**, 285-287.

Lin, C.H., Ding, H.Y., Kuo, S.Y., Chin, L.W., Wu, J.Y. and Chang, T.S. (2011): Evaluation of *in vitro* and *in vivo* depigmenting activity of raspberry ketone from *Rheum officinale*. Int. J. Mol. Sci., **12**, 4819-4835.

Nishigori, C., Aoyama, H., Ito, A., Suzuki, K., Suzuki, T., Tanemura,

A., Ito, M., Katayama, I., Oiso, N., Kagohashi, Y., Sugiura, S., Fukai, K., Funasaka, Y., Yamashita, T. and Matsunaga K. (2014): Rhododenol-induced leukoderma. Treatment guidelines for healthcare professionals (Dermatologists). Jpn. J. Dermatol., **124**, 285-303.

Nishimura, E.K. (2011): Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell Melanoma Res., **24**, 401-410.

Riley, P.A., Sawyer, B. and Wolf, M.A. (1975): The melanocytotoxic action of 4-hydroxyanisole. J. Invest. Dermatol., **64**, 86-89.

Riley, P.A. (1969a): Hydroxyanisole depigmentation: *in-vitro* studies. J. Pathol., **97**, 193-206.

Riley, P.A. (1969b): Hydroxyanisole depigmentation: *in-vivo* studies. J. Pathol., **97**, 185-191.

Riley, P.A. (1970): Mechanism of pigment-cell toxicity produced by hydroxyanisole. J. Pathol., **101**, 163-169.

Riley, P.A. (1971): Acquired hypomelanosis. Br. J. Dermatol., **84**, 290-293.

Robertson, A.R. (1977): The CIE 1976 color difference formulas. Corlor Res. Application., **2**, 7-11.

Seitz, J.C. and Whitmore, C.G. (1988): Measurement of erythema and tanning responses in human skin using a tri-stimulus colorimeter. Dermatologica, **177**, 70-75.

Staricco, R.J. and Pinkus, H. (1957): Quantitative and qualitative data on the pigment cells of adult human epidermis., J. Invest. Dermatol., **28**, 33-45.

Tayama, K. and Takahama, M. (2002): Depigmenting action of phenylhydroquinone, an O-phenylphenol metabolite, on the skin of JY-4 black guinea-pigs. Pigment Cell Res., **15**, 447-453.

van den Boorn, J.G., Picavet, D.I., van Swieten, P.F., van Veen, H.A., Konijnenberg, D., van Veelen, P.A., van Capel, T., Jong, E.C., Reits, E.A., Drijfhout, J.W., Bos, J.D., Melief, C.J. and Luiten, R.M. (2011): Skin-depigmenting agent monobenzone induces potent T-cell autoimmunity toward pigmented cells by tyrosinase haptenation and melanosome autophagy. J. Invest. Dermatol., **131**, 1240-1251.

Zhu, Y., Wang, S. and Xu, A. (2013): A mouse model of vitiligo induced by monobenzone. Exp. Dermatol., **22**, 499-501.

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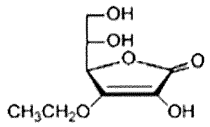
Key words: allergic contact dermatitis; cosmetics; 3-*o*-ethyl-L-ascorbic acid; skin-lightening agent; vitamin C ethyl.

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.

References

1 Nakagawa M, Kawai K, Kawai K. Contact allergy to kojic acid in skin care products. *Contact Dermatitis* 1995: **32**: 9–13.

2 Suzuki K, Yagami A, Matsunaga K. Allergic contact dermatitis caused by a skin-lightening agent, 5,5′-dipropylbiphenyl-2,2′-diol. *Contact Dermatitis* 2012: **66**: 51–52.

3 Cosmetic Ingredient Review Expert Panel. Final report of the safety assessment of L-ascorbic acid, calcium ascorbate, magnesium ascorbate, magnesium ascorbyl phosphate, sodium ascorbate, and sodium ascorbyl phosphate as used in cosmetics. *Int J Toxicol* 2005: **24** (Suppl. 2): 51–111.

4 Maeda K, Inoue Y, Nishikawa H, Miki S, Urusibata O, Miki T, Hatao M. Involvement of melanin monomers in the skin persistent UVA-pigmentation and effectiveness of vitamin C ethyl on UVA-pigmentation. *J Jpn Cosmet Sci Soc* 2003: **27**: 257–267 (in Japanese).

5 Inge S, Goossens A. Allergic contact dermatitis caused by ascorbyl tetraisopalmitate. *Contact Dermatitis* 2011: **64**: 237–244.

6 Belhadajali H, Giordano-Labadie F, Bazex J. Contact dermatitis from vitamin C in a cosmetic anti-aging cream. *Contact Dermatitis* 2001: **45**: 317.

7 Metz J, Hundertmark U, Pevny I. Vitamin C allergy of the delayed type. *Contact Dermatitis* 1980: **6**: 172–174.

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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．

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

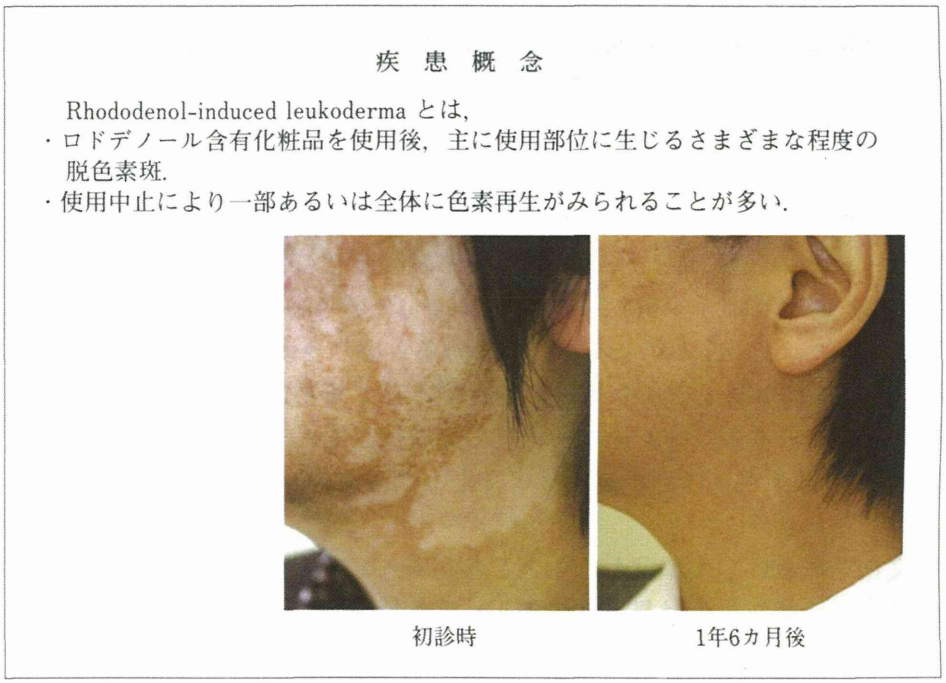

図1　RD誘発性脱色素斑の疾患概念と典型例．初診時およびRD含有化粧品中止後1年6カ月後の回復時臨床像．

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ロドデノール含有化粧品

対象製品名
【株式会社カネボウ化粧品】
<カネボウブランシール スペリア>
・ホワイトディープ クリアコンディショナー 全7品
・ホワイトディープ ミルキィコンディショナー 全3品
・ホワイトディープ ナイトコンディショナー 全4品
・ホワイトディープ マスク
・ホワイトディープ UV デイプロテクター
<suisai>
・ホワイトニングエッセンス
<トワニー>
・エスティチュードホワイト ローション 全6品
・エスティチュードホワイト UV プロテクトセラム
・エスティチュードホワイト クリアタイトマスク
・センチュリー ザ・ローション 全2品
<インプレス>
・IC ホワイトローション 全2品
・IC ホワイトエマルジョン 全2品
・IC ホワイトフィットマスク 3D
・グラン ミュラローション
<アクアリーフ>
・MCT ホワイトニングエッセンス

【株式会社リサージ】
<リサージ>
・ホワイト スキンメインテナイザー 全8品
・ホワイト ホワイトニング リペアクリーム
・ホワイト トライアルセット 全4品
・ボーテ サーキュリード a
【株式会社エキップ】
<RMK>
・スキンチューナー ブライトニング 全2品
・インテンシブ ブライトニング エッセンス
<SUQQU>
・ホワイトニング リペア エッセンス
・ホワイトニング ローション
・ホワイトニング バリア エマルジョン

✓上記8ブランド，54製品，2008年から販売され，国内で約25万人が利用
✓海外で約10万人が利用
✓年間売上高50億円

（上段左から）インプレス：IC ホワイトローション，トワニー：エスティチュードホワイト ローション，
カネボウブランシール スペリア：ホワイトディープ クリアコンディショナー，suisai：ホワイトニング エッセンス
（下段左から）リサージ：ホワイト スキンメインテナイザー，RMK：インテンシブ ブライトニング エッセンス，
SUQQU：ホワイトニング リペア エッセンス，アクアリーフ：MCT ホワイトニングエッセンス

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

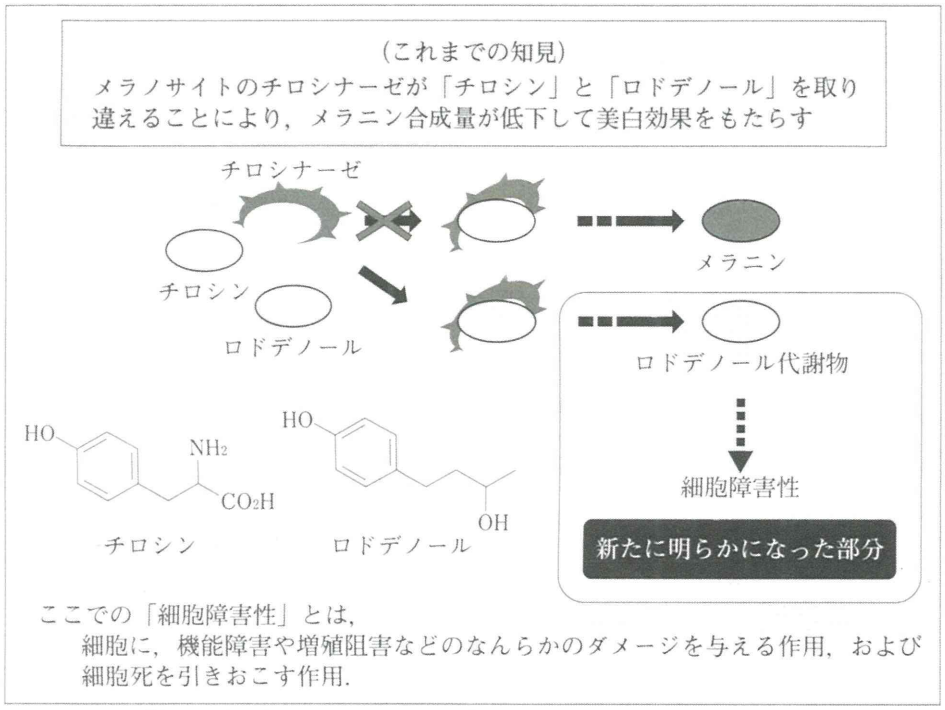

図3　RDの「美白」作用のメカニズムと細胞障害性

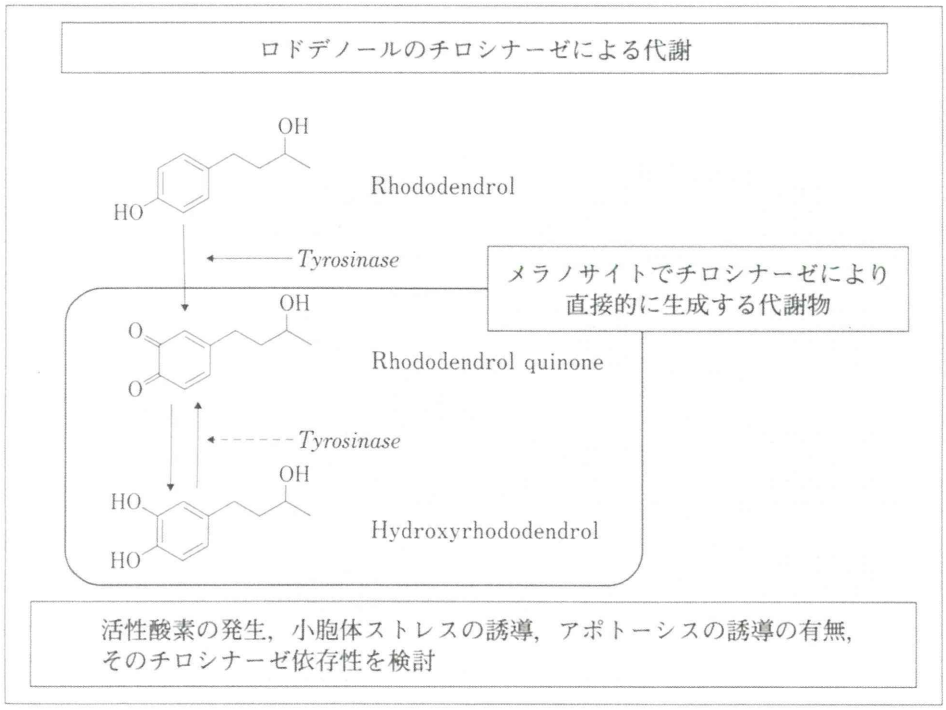

図4　RDのチロジナーゼによる代謝

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