[2] McGuire J, Hendee J. Biochemical basis for depigmentation of skin by phenolic germicides. J Invest Dermatol 1971;57:256–61.
[3] Jimbow K, Obata H, Pathak MA, Fitzpatrick TB. Mechanism of depigmentation by hydroquinone. J Invest Dermatol 1974;62:436–49.
[4] Ito S, Ojika M, Yamashita T, Wakamatsu K. Tyrosinase-catalyzed oxidation of rhododendrol produces 2-methylchromane-6,7-dione, the putative ultimate toxic metabolite: implications for melanocyte toxicity. Pigment Cell Melanoma Res 2014.
[5] Sasaki M, Kondo M, Sato K, Umeda M, Kawabata K, Takahashi Y, et al. Rhododendrol, a depigmentation-inducing phenolic compound, exerts melanocyte cytotoxicity via a tyrosinase-dependent mechanism. Pigment Cell Melanoma Res 2014.
[6] Kasamatsu S, Hachiya A, Nakamura S, Yasuda Y, Fujimori T, Takano K, et al. Depigmentation caused by application of the active brightening material, rhododendrol, is related to tyrosinase activity at a certain threshold. J Dermatol Sci 2014.
[7] Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 2007;282:24131–45.
[8] Lee AS. The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. Methods 2005;35:373–81.
[9] Boissy RE, Manga P. On the etiology of contact/occupational vitiligo. Pigment Cell Res 2004;17:208–14.
[10] Westerhof W, d'Ischia M. Vitiligo puzzle: the pieces fall in place. Pigment Cell Res 2007;20:345–59.

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Letter to the Editor

An immune pathological and ultrastructural skin analysis for rhododenol-induced leukoderma patients

Keywords:
Rhododenol-induced leukoderma; Histopathological and ultrastructural analyses of the skin; Immune-competent cells

A B S T R A C T

As reported in the mass media on July 2013, numerous consumers who had used the cosmetic ingredient containing rhododendrol (4-(4-hydroxyphenyl)-2-butanol, Trade name; rhododenol), which is a melanin inhibitor isolated from Acer nikoense Maxim, released from Kanebo Cosmetics Inc. (Tokyo, Japan) noticed leukoderma patches on their face, neck and hands. We have experienced 32 cases that developed leukoderma after using such cosmetics so far and skin biopsy samples in some cases were obtained from both leukoderma and pigmented lesions. A histopathological analysis for skin lesions obtained from such patients notably showed basal hypo-pigmentation, melanin incontinence, and remaining melanocytes in most patients which is not relevant in vitiligo vulgaris. Subsequently, we comprehensively carried out immunohistochemical analyses of immune-competent cells infiltration to assess the effect of the cellular immune response to inducible hypopigmentation. Furthermore, detailed morphological observations performed by electron-microscopy notably showed the presence of melanocytes with only a small number of melanosomes, dermal fibroblasts containing melanosome globules and melanophages whereas no damage associated with melanosome transfer and the basal layer apparatus. These findings provide a cue to diagnose as rhododenol-induced leukoderma differentiate from vitiligo vulgaris and for rhododendrol to induce local immunity in addition to melanocyte damage.

Rhododenol-induced leukoderma was found to have occurred in approximately 2% of the consumers who had used the cosmetics containing rhododendrol and the total number of such patients is estimated to be more than 9000 individuals. Since the products in question produced by the Kanebo corporation had been sold on the Asian market in not only Japan, but also in Korea and Taiwan, the associated health hazard is thus considered to be a serious and widespread problem. Recent research has unveiled the biochemical and physiological mechanism of melanocyte cell damage induced by rhododenol-metabolites after tyrosinase reaction [1,2]. Soon after rhododendrol was oxidated by endogeneous tyrosinase in cytoplasm, the metabolite form could thus become toxic to melanocytes due to endoplasmic reticulum and oxidative stress production [2] in addition to NO production [3], however, it is not yet investigated whether lesional immune reaction could affect the pathogeny of rhdodenol-induced leukoderma. Previously, we analyzed histopathological alteration and the infiltration of immune-competent cells in vitiligo skin followed by an investigation of the local immune milieu on melanocyte dysfunction and disappearance in the occurrence and maintenance of vitiligo vulgaris [4–7]. In this communication, we obtained hypopigmented skin specimens from patients and carried out an immune-pathological analysis of 32 lesions and an ultrastructural analysis of 6 lesions to assess morphological change of skin component cells and local cellular immune reaction. Comprehensive infiltrating cells number was summarized in Table 1. A small number of melanosomes were found remaining in the basal layer while many were present in the dermis, along with melanin incontinentia

Table 1
Results of an immunohistochemical analysis for immune competent cell infiltration. The data represent the mean number of infiltrating cells calculated by three independent physicians under ×100 magnification. A statistical analysis for comparison purposes was performed by using the unpaired *t*-test and the *p*-value was calculated in comparison with that obtained from normal skin.

	MelanA	S100$^+$ MITF$^+$	CD3	CD8	CD4	CD4$^+$ IL17A$^+$	Foxp3$^+$	CD20	CD56
Rhodo-induced leukoderma ($n=32$)	$9.5 \pm 9.0^{**}$	$5.0 \pm 5.4^{**}$	$68.8 \pm 39.7^{*}$	$32.7 \pm 19.2^{*}$	$41.1 \pm 17.5^{**}$	$8.3 \pm 5.7^{*}$	$17.3 \pm 10.1^{*}$	1.5 ± 2.3	1.5 ± 3.3
Nonsegmental vitiligo vulgaris ($n=6$)	$1.7 \pm 3.5^{**}$	$1.2 \pm 1.7^{**}$	$58.2 \pm 27.9^{*}$	$39.5 \pm 22.7^{**}$	$19.7 \pm 5.4^{*}$	$8.9 \pm 4.7^{*}$	8.6 ± 4.1	3.7 ± 3.2	4.5 ± 1.2
Normal ($n=3$)	39.3 ± 3.1	32.1 ± 5.9	15.2 ± 2.5	7.8 ± 2.4	12.3 ± 2.1	8.5 ± 1.0	7.9 ± 3.2	5.88 ± 1.1	0

* $p < 0.01$.
** $p < 0.05$.

(Fig. 1a). Lymphocytes were found invading with a focus on the upper dermis in rhododenol-induced leukoderma lesions and the mean number of CD4$^+$ T cells was significantly more abundant compared to that of nonsegmental vitiligo lesions (Fig. 1b). Although the MITF or MelanA-positive melanocyte count had significantly declined compared to normal skin, complete disappearance was observed in only 4 among 31 cases (Fig. 1c). Although recent literatures implicated the toxicity of rhododendrol metabolite to melanocytes in vitro, we unexpectedly observed little structural damage of lesional remaining melanocytes (Fig. 1d). Significant number of mature melanosomes was arranged on the periphery and no vacuolization and no disturbance of melanin transfer were detected [8], considering elimination of severely affected melanocytes by phagocytosis (Fig. 1d). The basement membrane was observed under an electron microscope in order to clarify the cause of melanin dripping; although destruction of the basement

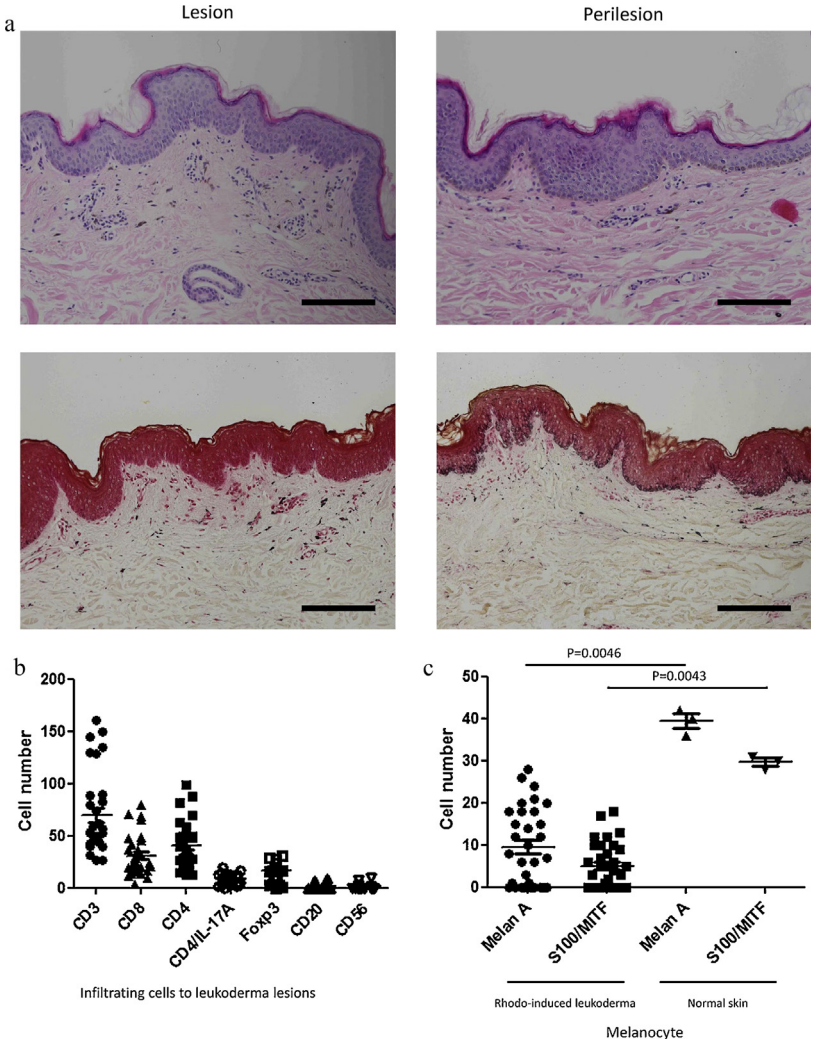

Fig. 1. (a) Histological findings of rhododenol-induced leukoderma skin specimens. The upper and lower rows show H and E staining and Masson–Fontana staining, respectively. Bar indicates 100 μm. (b) Dot plot of the immune-competent cells' number infiltrated into rhododenol-induced leukoderma lesions. Notably, T cell infiltration was predominant rather than the infiltration of NK cells and B cells. (c) Dot plot analysis for the number of melanocytes remaining on rhododenol-induced leukoderma lesions. While the number of remaining melanocytes varied between each patient, only a few cases lost MelanA$^+$ or MITF$^+$ melanocytes. (d) An ultrastructural feature for melanocyte remaining on leukoderma lesion. Visible melanosomes were mostly in stage IV and appeared to be regularly arranged along with cell membrane (left). Intracellular organelles including mitochondria and rough endoplasmic reticulum were not notably altered. Left and right panels indicate ×10,000 and ×20,000 original magnification, respectively. (e) An ultrastructural analysis for dermal fibroblasts which phagocyte melanosomes in various stages. A dermal fibroblast containing melanosomes was detected just below the basal membrane (upper left). The melanosomes were located in the intracellular lysosome, thus indicating phagocyte and enzymatic digestion (upper right). A higher magnification view shows no alteration of hemidesmosomes and the basal membrane (lower left). Upper left, upper right, and lower left panels indicate ×2000, ×15,000, and ×6000 original magnification, respectively.