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Smoking is Associated with the Severity of Rhododendrol-induced Leukoderma and with the Occurrence of Leukomelanoderma

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Abstract

Background

Rhododendrol (RD) is a skin whitening ingredient that was developed in Japan. Among the 800000 users of RD-containing cosmetics, 20000 patients developed localized leukoderma (RD-induced leukoderma). Forty-two % of those users showed perilesional hyperpigmentation (leukomelanoderma), and 14% of them were associated with vitiligo vulgaris afterwards.

Methods

For this retrospective cohort study, we abstracted data from our dermatology medical records of 101 patients who developed leukoderma after using the cosmetics containing RD from July 2013 to December 2014. Age, BMI, the number of RD-containing products they used, smoking history, and depigmentation scores at their baseline visit as well as blood test data for anti-nuclear and/or anti-thyroid antibodies were analyzed. Multivariable logistic regression and linear regression were used for analyses of leukomelanoderma, vitiligo vulgaris and characteristics at the baseline visit.

Results

Age, the number of RD-containing products used, BMI, anti-nuclear, and anti-thyroid antibodies were not significantly correlated with the presence of leukomelanoderma, but it appeared that leukomelanoderma was more likely to occur in patients with current smoking. In addition, smokers showed a significant increase in their depigmentation score at the baseline visit.

Conclusions

Our study demonstrates that smoking is associated with the severity of RD-induced leukoderma and the occurrence of leukomelanoderma.

Key Words: Rhododendrol; Leukoderma; Vitiligo vulgaris; Leukomelanoderma; Smoking

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Introduction

Rhododendrol (RD, 4-[4-hydroxyphenyl]-2-butanol) is a skin whitening ingredient that was developed in 2006 by Kanebo Cosmetics. Cosmetics containing RD were on sale from 2008 in Japan. However, it turned out that among the users of RD-containing cosmetics, a considerable number of users developed leukoderma in 2013. As many as 20000 users, approximately 2% of the 800000 users of RD-containing cosmetics, developed RD-induced leukoderma. After discontinuing use of those cosmetics, most leukodermas showed spontaneous repigmentation. However, some users noted that their leukodermas still persisted for several years, and new leukodermas appeared in non-RD exposed skin areas, suggesting the development of vitiligo vulgaris[1].

The Japanese Dermatological Association (JDA) established a Special Committee on the Safety of Cosmetics Containing Rhododendrol and reported a nationwide epidemiological survey[2-4] after developing a scoring system to evaluate the severity of leukodermas. The median depigmentation score at the baseline visit was 6 (range 0-48) among the 1315 patients whose questionnaire of the second nationwide survey was available, and most of those were at a relatively lower score[3]. Hyperpigmentation in depigmented or surrounding area was observed in 42% of 1235 patients whose responded to the questionnaire, and one third of them improved afterward[3].

Fourteen % of patients who were subjects of the third nationwide survey observed, expansions of the areas of depigmentation or the development of vitiligo vulgaris at non-exposed areas[4]. RD was developed and was thought to reduce melanin synthesis, as a competitive inhibitor of tyrosinase, the enzyme critical to melanin synthesis[5,6]. RD itself is degraded by tyrosinase and gives rise to harmful metabolites that are toxic to melanocytes[7]. In susceptible subjects, the damaged melanocytes induced T-cell responses which activated site-specific cytotoxic lymphocytes (CTLs). Those, CTLs may cause the spread of depigmentation to non-exposed sites[8].

Cigarette smoke contains a variety of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals and other chemicals that increase the burden of oxidative stress[9]. Oxidative stress induced by ROS has been implicated in the pathogenesis of autoimmune diseases because it may damage autoimmune target cells and produce novel antigens[10]. It has also been reported that vitiligo vulgaris develops due to the overproduction of ROS in melanocytes[11].

In this retrospective cohort study, we collected data of patients who visited our institution for the treatment of RD-induced leukoderma. Their data was analyzed to identify risk factors of the severity of RD-induced leukoderma and the occurrence of perilesional hyperpigmentation (leukomelanoderma).

Methods

Patients

After approval by the Institutional Review Board of Osaka City University Medical School (#3810), we assembled a retrospective cohort of 101 patients with RD-induced leukoderma, who had visited our Dermatology Department from July 2013 to December 2014. From their medical records, we collected their age, BMI, the number of RD-containing products used, smoking history, and depigmentation scores at their baseline visit. Blood tests were conducted to assess whether they had anti-nuclear and/or anti-thyroid antibodies at their baseline visit. In addition, whether the patients developed vitiligo vulgaris or hyperpigmentation at surrounding areas (i.e. leukomelanoderma) during the course of treatment was also recorded.

Evaluation of the depigmentation score and leukomelanoderma

The Depigmentation score as defined by the JDA was calculated as the sum of the six parts of individual area scores: forehead, periorbital areas, cheeks (right and left sides), nose and mouth, neck and hands. Each individual area score was the multiplication of the degree (i.e. complete depigmentation: 2, incomplete depigmentation: 1) and the area of depigmentation: 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (76%-100%). The maximum total depigmentation score was 48. The presence or absence of leukomelanoderma was visually assessed independently by two dermatologists using photographs.

Statistical analysis

We assessed the factors associated with 1) the severity of the leukoderma, which is quantified by the depigmentation score at the baseline visit, 2) the occurence of leukomelanoderma, and 3) the occurrence of vitiligo vulgaris. Multivariable logistic regression was used for binomial outcomes of the occurrence or non-occurrence of leukomelanoderma as well as vitiligo vulgaris during the treatment course. Multivariable linear regression was used for continuous outcome of the first depigmentation score to assess the effects of various risk factors including age, smoking history, number of RD-containing roducts used, BMI, anti-nuclear, and anti-thyroid antibodies. The depigmentation score at the baseline visit was natural log-transformed to provide normality in the residuals, and coefficients obtained by the regression model were back-transformed indicating the % increase in the first depigmentation score by an interquartile range (IQR) increase in corresponding covariates. The non-linearity of continuous risk factor variables was assessed using a restricted cubic spline. No significant non-linearity was observed. Missing data were imputed using the multiple imputation method because the exclusion of patients who had missing data might cause a selection bias. Demographic and clinical characteristics of the patients were presented using median and IQR for continuous variables and frequencies and percentages for categorical variables. All statistical analyses used a two tailed significance level of 0.05. All statistical calculations were performed using R software, version 3.5.1 (www.r-project.org).

Results

Characteristics of the patients

We enrolled 101 patients who developed leukoderma after using cosmetics containing RD, and their demographics are listed in Table 1. It shows median or percentage value of each risk factor that we hypothesize, depigmentation score, and complication with vitiligo vulgaris and leukomelanoderma. Regarding smoking history, twelve of patients (12%) had it, however, there was no former-smoker but current-smoker.

Depigmentation scores at the baseline visit

Histogram plots of depigmentation scores at the baseline visit are shown in Figure 1. The median value of the depigmentation score at the baseline visit was 14 (IQR, 4-32) (Table 1).

Development of leukomelanoderma and vitiligo vulgaris

Results of the multivariable logistic regression are shown in Figure 2. We analyzed the association of leukomelanoderma and vitiligo vulgaris with each of the candidate risk factors. Age, number of RD-containing products used, BMI, anti-nuclear, and anti-thyroid antibodies were not significantly correlated with the outcome, but it appeared that leukomelanoderma was more likely to occur in patients with current smoking (odds ratio 21.53, 95% CI 2.39-193.78; p=0.006).

Table 1. Demographic and clinical characteristics of patients at the baseline visit

Variable	N=101
Age in years, median (IQR)	60 (48, 70)
Female, n (%)	101 (100)
Smoking, n (%)	12 (12)
Number of products, median (IQR)	9.5 (5, 17)
BMI, median (IQR)	21.6 (19.58, 23.48)
Positive for anti-nuclear antibody, n (%)	11 (12)
Positive for anti-thyroid antibody, n (%)	26 (28)
Depigmentation score, median (IQR)	14 (4, 32)
Complication of vitiligo vulgaris, n (%)	10 (10)
Complication of leukomelanoderma, n (%)	48 (48)

IQR, interquartile range.

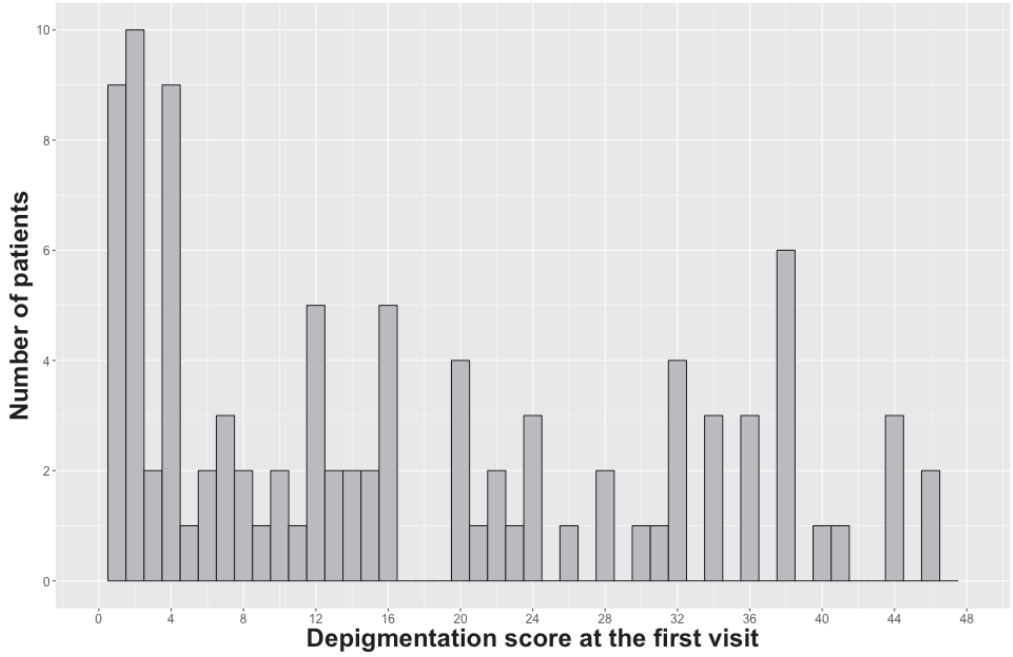

Figure 1. Histograms of depigmentation scores at the baseline visit (n=101). Most patients presented with a relatively low depigmentation score.

The effect of smoking

As indicated by linear regression analysis (Table 2), patients who have smoking history had significant increases in the depigmentation score at the baseline visit (coefficient 1.239, p=0.012). The other factors such as age, the number of RD-containing products used, BMI, anti-nuclear, and anti-thyroid antibodies did not appear to show a significantly different effect on the first depigmentation score at the baseline visit. Box plots were used to compare the depigmentation scores at the baseline visit between smokers and non-smokers (Fig. 3). That analysis showed that the median of the depigmentation score at the baseline visit was significantly higher among patients with a smoking history (median score 26 for the smokers, 13 for non-smokers, p=0.03).

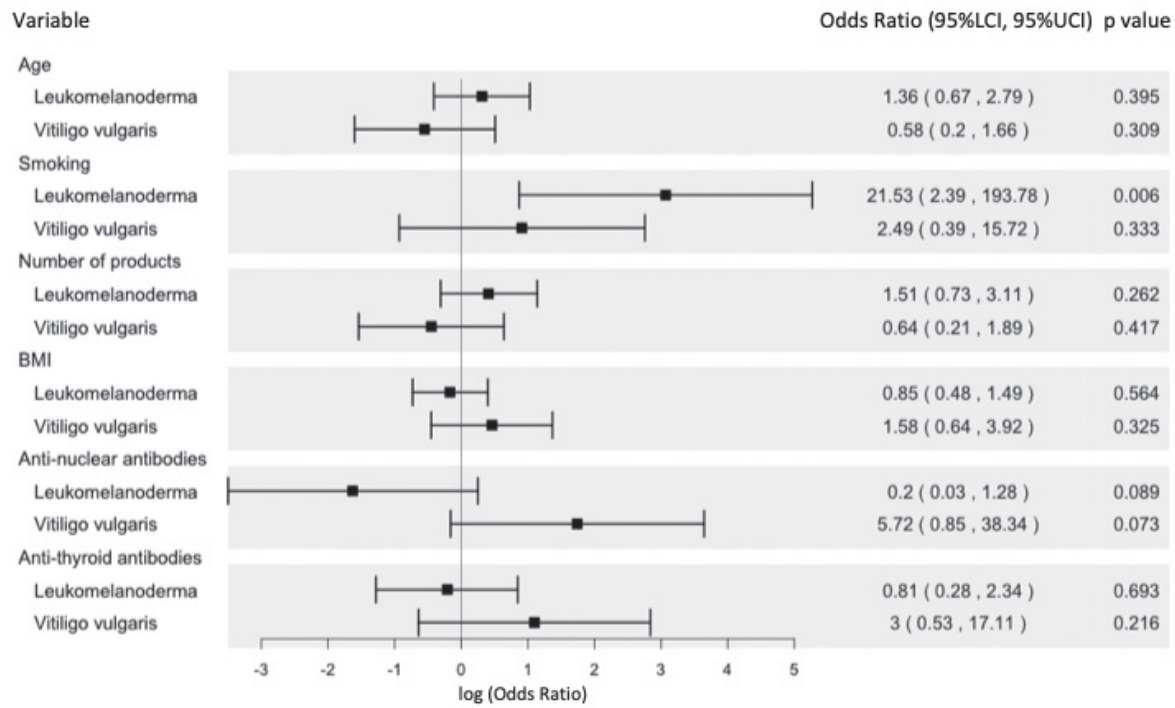

Figure 2. Result of multivariable logistic regression for leukomelanoderma and vitiligo vulgaris including age, smoking, number of rhododendrol (RD) -containing products used, BMI, anti-nuclear, and anti-thyroid antibodies. Odds ratios reflect a comparison between the 25th and the 75th percentile values for each variable. Age, smoking, number of RD-containing products used, BMI, anti-nuclear, and anti-thyroid antibodies were not significantly correlated with leukomelanoderma and/or vitiligo vulgaris, but leukomelanoderma was likely to occur in patients who had a smoking history (odds ratio 21.53, 95% CI 2.39-193.78; p=0.006).

Table 2. Linear regression analysis of the effect of each factor on the depigmentation scores at the baseline visit

Variable	Coefficient	95% CI	p value
Age (IQR: 48-70)	1.295	[0.897, 1.869]	0.165
Smoking	1.239	[1.239, 5.505]	0.012
Number of products (IQR: 5-17)	1.004	[0.856, 1.177]	0.960
BMI (IQR: 19.6-23.5)	0.915	[0.685, 1.223]	0.546
Positive for anti-nuclear antibody	0.788	[0.387, 1.644]	0.522
Positive for anti-thyroid antibody	1.258	[0.695, 2.277]	0.444

Coefficients are indicated as % increase in depigmentation scores at the baseline visit by IQR increase in the corresponding covariates. IQR, interquartile range.

Discussion

This study shows that smoking is associated with the increase of the depigmentation score at the baseline visit and also the development of leukomelanoderma. To the best of our knowledge, only one clinical study exists regarding the risk factors related to RD-induced leukoderma[12]. Patients with a history of atopic dermatitis or patients who had a higher depigmentation score at the baseline visit significantly developed vitiligo vulgaris after the RD-induced leukoderma[12]. However, this is the first report referring to an association between smoking and RD-induced leukoderma.

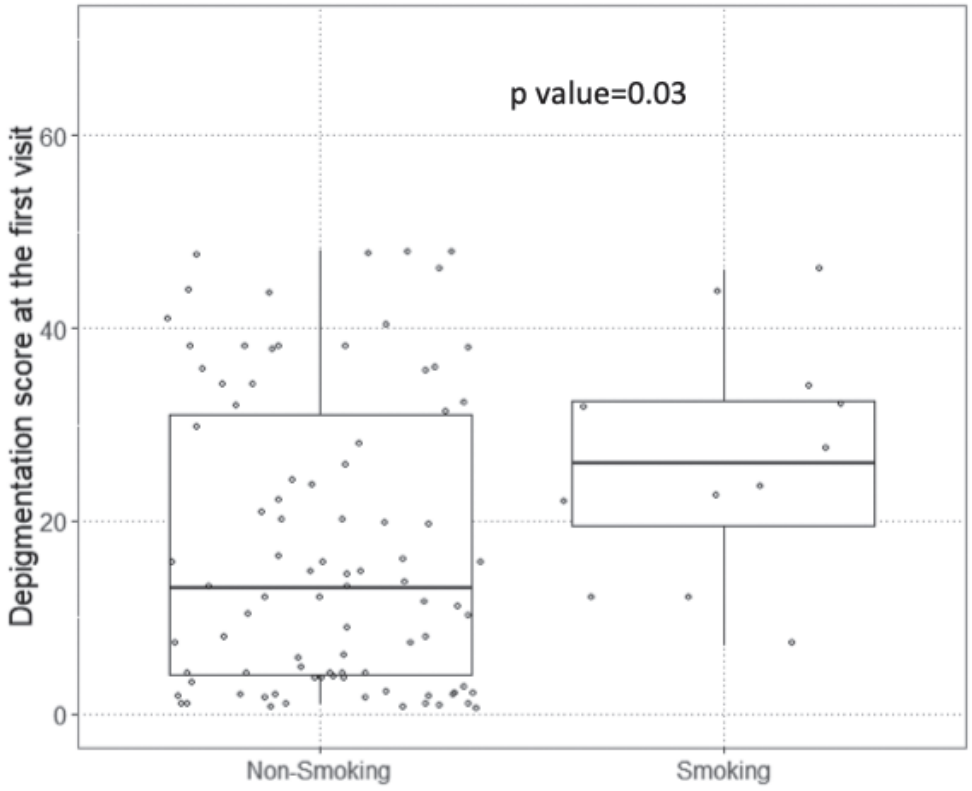

Figure 3. Box plots of depigmentation scores at the baseline visit by smokers and non-smokers. Mann-Whitney U test showed the median depigmentation score of smokers at the baseline visit is higher than non-smokers (median score 26 for smokers, 13 for non-smokers, p=0.03).

RD is a competitive inhibitor of tyrosinase and reduces melanin production, but it is also cytotoxic to human melanocytes[5]. One of the mechanisms for its cytotoxicity is the effect of RD-quinone through binding with sulfhydryl proteins, and the other is the pro-oxidant activity of RD-derived melanins that leads to oxidative stress[13]. Within human melanocytes, tyrosinase oxidizes RD to RD-quinone and RD-cyclic-quinone which is cytotoxic to melanocytes. When these toxic substances combine with thiol-containing chemicals such as glutathione (GSH) and cysteine (Cys), they will be detoxified to RS-catechol and RS-cyclic-catechol[7,14,15]. RD-catechol and RD-cyclic catechol which are metabolites of RD-quinone generate superoxide in the process of their oxidization. It has also been reported that superoxide dismutase (SOD) oxidizes superoxide to hydrogen peroxide (H_2O_2) and oxygen. H_2O_2 which is one type of ROS, is detoxified by combining with a thiol group[14]. In smokers, the plasma levels of GSH and Cys have been shown to be significantly lower than in nonsmokers[16]. Thus, metabolites of RD and ROS are supposed to be less detoxified in smokers, given that GSH and Cys are exhausted from the detoxification of smoking-related chemicals.

Leukomelanoderma is a combined form of dyspigmentation, composed of speckled pattern of hyperpigmentation along with wider areas of hypopigmentation (Fig. 4). Leukomelanoderma can be caused by the medication history, such as oral thiazide-based diuretics[17,18], and by the application of hydroquinone[19]. The mechanism of leukomelanoderma induced by medication may be due to sun exposure, the color of the skin and the genetic predisposition, however, none of these are obvious[18].

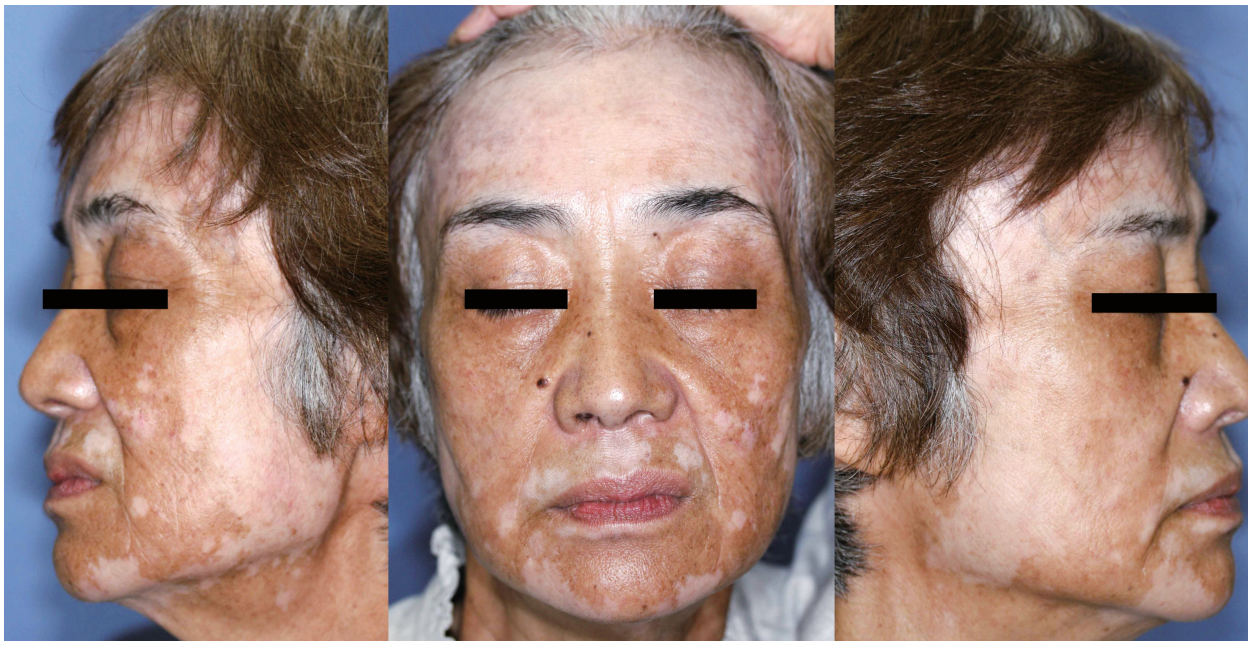

Figure 4. Clinical appearance of leukomelanoderma. Speckled hyper- and hypo-pigmentation are intermixed on a relatively wide range of the skin. This patient was 69-year-old woman who had a smoking history at the first visit. Her depigmentation score was 28.

In regard to hydroquinone, the combination of post-inflammatory pigmentation induced by contact dermatitis and hydroquinone-induced leukoderma has been postulated[19]. In fact, contact dermatitis due to RD was suggested by positive patch testing, as reviewed by the first epidemiological report from the JDA[2]. The presence of leukomelanoderma considerably lowers the quality of life of patients. It has been reported that perilesional hyperpigmentation was seen in 42% of patients with RD-induced leukoderma[3], and in this study cohort, 48% of the patients had leukomelanoderma.

Tobacco smoking induces several undesirable characteristics of skin aging such as wrinkles and increased skin pigmentation[20-23]. Melanin indices of the skin among current smokers are higher when compared with never and former smokers[23]. Human epidermal melanocytes are activated by water-soluble tobacco smoke through the Wnt/β-catenin signaling pathway and produce more melanin *in vitro*[24].

A limitation of this study is that the number of patients who smoked was small. Although the percentage (12%) was higher than the women's value (8.2%) of a national health and nutrition survey in 2016 in Japan, it may be difficult to generalize our results because the sample size was small.

In conclusion, our study has shown that smoking induces more severe RD-induced leukoderma. The depigmentation score at the baseline visit of smokers is higher, suggesting that cytotoxic substances may accumulate in the course of the metabolism of RD because of the low levels of GSH and Cys in the plasma[16]. In addition, smoking may lead to a higher risk of leukomelanoderma probably due to post-inflammatory pigmentation caused by contact dermatitis.

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All authors have no COI to declare regarding the present study.

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References

1. Nishigori C, Aoyama Y, Ito A, et al. Guide for medical professionals (i.e., dermatologists) for the management of Rhododenol-induced leukoderma. J Dermatol 2015;42:113-128.
2. Aoyama Y, Ito A, Suzuki K, et al. The first epidemiological report of Rhododendrol-induced leukoderma in Japan based on a nationwide survey. The Japanese Journal of Dermatoology 2014;124:2095-2109. (In Japanese)
3. Suzuki K, Aoyama Y, Ito A, et al. The second epidemiological report of Rhododendrol-induced leukoderma in Japan based on a nationwide survey. The Japanese Journal of Dermatoology 2014;124:3125-3142. (In Japanese)
4. Ito A, Aoyama Y, Suzuki K, et al. The third epidemiological report of Rhododendrol-induced leukoderma in Japan based on a nationwide survey. The Japanese Journal of Dermatoology 2015;125:2401-2014. (In Japanese)
5. Sasaki M, Kondo M, Sato K, et al. Rhododendrol, a depigmentation-inducing phenolic compound, exerts melanocyte cytotoxicity via a tyrosinase-dependent mechanism. Pigment Cell Melanoma Res 2014;27:754-763.
6. Iwadate T, Kashiwakura Y, Masuoka N, et al. Chemical synthesis and tyrosinase inhibitory activity of rhododendrol glycosides. Bioorg Med Chem Lett 2014;24:122-125.
7. Okura M, Yamashita T, Ishii-Osai Y, et al. Effects of rhododendrol and its metabolic products on melanocytic cell growth. J Dermatol Sci 2015;80:142-149.
8. Tokura Y, Fujiyama T, Ikeya S, et al. Biochemical, cytological, and immunological mechanisms of rhododendrol-induced leukoderma. J Dermatol Sci 2015;77:146-149.
9. van der Toorn M, Rezayat D, Kauffman HF, et al. Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. Am J Physiol Lung Cell Mol Physiol 2009;297:L109-114.
10. Di Dalmazi G, Hirshberg J, Lyle D, et al. Reactive oxygen species in organ-specific autoimmunity. Auto Immun Highlights 2016;7:11.
11. Xie H, Zhou F, Liu L, et al. Vitiligo: How do oxidative stress-induced autoantigens trigger autoimmunity? J Dermatol Sci 2016;81:3-9.
12. Yoshikawa M, Sumikawa Y, Hida T, et al. Clinical and epidemiological analysis in 149 cases of rhododendrol-induced leukoderma. J Dermatol 2017;44:582-587.
13. Ito S, Wakamatsu K. Biochemical mechanism of rhododendrol-induced leukoderma. Int J Mol Sci 2018;19:552.
14. Ito S, Ojika M, Yamashita T, et al. 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;27:744-753.
15. Ito S, Gerwat W, Kolbe L, et al. Human tyrosinase is able to oxidize both enantiomers of rhododendrol. Pigment Cell Melanoma Res 2014;27:1149-1153.
16. Moriarty SE, Shah Jh, Lynn M, et al. Oxidation of glutathione and cysteine in human plasma associated with smoking. Free Radic Biol Med 2003;35:1582-1588.
17. Seishima M, Shibuya Y, Kato G, et al. Photoleukomelanoderma possibly caused by etretinate in a patient with psoriasis. Acta Derm Venereol 2010;90:85-86.
18. Masuoka E, Bito T, Shimizu H, et al. Dysfunction of melanocytes in photoleukomelanoderma following photosensitivity caused by hydrochlorothiazide. Photodermatol Photoimmunol Photomed 2011;27:328-330.
19. Yanagishita-Nakatsuji S, Fukai K, Ohyama A, et al. Probable allergic contact dermatitis from hydroquinone presenting as leukomelanoderma: report of two cases. J Dermatol 2017;44:e330-e331.
20. Yin L, Morita A, Tsuji T. Skin aging induced by ultraviolet exposure and tobacco smoking: evidence from epidemiological and molecular studies. Photodermatol Photoimmunol Photomed 2001;17:178-183.
21. Leung WC, Harvey I. Is skin ageing in the elderly caused by sun exposure or smoking? Br J Dermatol 2002; 147:1187-1191.
22. Okada HC, Alleyne B, Varghai K, et al. Facial changes caused by smoking: a comparison between smoking and nonsmoking identical twins. Plast Reconstr Surg 2013;132:1085-1092.
23. Tamai Y, Tsuji M, Wada K, et al. Association of cigarette smoking with skin colour in Japanese women. Tob Control 2014;23:253-256.
24. Nakamura M, Ueda Y, Hayashi M, et al. Tobacco smoke-induced skin pigmentation is mediated by the aryl hydrocarbon receptor. Exp Dermatol 2013;22:556-558.