enzyme (Figure 1B). *E. coli* FECH was also examined for the conversion activity but was ineffective on the activity (data not shown). Among the enzymes examined, yeast FECH showed the highest activity.

It is known that mammalian FECH includes an iron−sulfur cluster at the carboxyl terminal. At present, the roles of the cluster are not fully understood. This cluster could play an important role in mammalian FECH activity.[26,27] The cluster can also be found in FECH from some kinds of bacteria and yeast *Schizosaccharomyces pombe*,[28] but its role is not clear. We compared the stability of the cluster-free FECH of the bacterium *T. thermophilus*, the yeast *S. cerevisiae*, and the porcine enzyme containing the cluster. Although the iron−sulfur cluster could not be related to the ability of the reverse and conversion reactions of FECH, the differences in stability of bacterial, mammalian, and yeast FECH could be related to the presence of the cluster. Oxygen, nitric oxide, and various chemicals can easily destroy the iron−sulfur cluster.[26] This was supported by the observations that the formation of Zn-protoporphyrin was decreased by the addition of nitrite in the processing of dry-cured ham.[17,21] Therefore, the mammalian FECH was unstable as compared with those of yeast and bacteria. The yeast FECH was quite stable with high activity (Figure 1B). Thus, yeast FECH can be the model supplement enzyme to obtain a high yield in the conversion of Zn-protoporphyrin from heme.

Ascorbic acid has been used as a preserving additive in meat products.[29] As shown previously, reducing systems can play a vital role in the reverse and conversion reactions of FECH. NADH-cytochrome b_5 reductase (metmyoglobin reductase) can reduce heme to enhance the reverse reaction.[11] In vitro, ascorbic acid and cysteine showed the same effect as NADH-cytochrome b_5 reductase on the reduction of ferric ions to ferrous ions, and then, the FECH can attack to remove ferrous ions in heme. In addition, ascorbic acid as well as cysteine at 6 mM can highly promote the reverse and conversion activities of FECH (Figure 3A). The enhancing ability of ascorbic acid on the formation of Zn-protoporphyrin found in this study is in agreement with another finding[18] that ascorbic acid can promote the formation of the pigments of dry-cured ham. The decrease of FECH activity at a higher concentration of ascorbic acid can be explained by the decrease of pH value. On the other hand, the formation of Zn-protoporphyrin in meat via the conversion reaction occurred without any addition of exogenous reductants, showing that some reducing systems in meat are present at a significant level or can be derived from some kinds of bacteria. The endogenous reductants such as ascorbic acid, glutathione, and nicotine nucleotides in meat can help the endogenous FECH-dependent occurrence of iron-removal and the zinc ion-insertion reactions of heme in meat during the processing of ham.

Other investigators[17,18] reported that the treatment of meat with NaCl improved the formation of Zn-protoporphyrin in dry-cured ham. A similar ability of salts was also found in the reaction system using meat extracts as FECH sources.[18] In these studies, because zinc ions and protoporphyrin were added to the reaction mixture, the formation of Zn-protoporphyrin could only occur via the forward reaction. In contrast, the present study showed that NaCl did not have any effects on the formation of Zn-protoporphyrin via the reverse and conversion reactions from hemoproteins catalyzed by FECH. The reason for this difference is unclear, but it is possible that the enhancement of the zinc-insertion reaction by NaCl contributed to different enzyme sources and experimental conditions. The other possibility is that the NaCl in ham maintains suitable growing conditions for yeast and some kinds of bacteria, which can enhance the formation of Zn-protoporphyrin in dry-cured ham.[8] However, the formation of Zn-protoporphyrin slightly decreased in the antibiotic-treated samples,[14] indicating that bacteria showed minor roles in the conversion reaction of Zn-protoporphyrin in dry-cured ham processing. Otherwise, it is possible that the addition of NaCl to meat during ham processing can prevent the growth of spoiling bacteria.[3]

Zinc ions can compete with iron in the insertion of divalent metal ions to protoporphyrin to form the corresponding metalloporphyrin.[30] In the case of reverse and conversion reactions of heme, the amount of protoporphyrin is lower than that of Zn-protoporphyrin in the same reaction condition, indicating that zinc ions can enhance the removal reaction to remove the protoporphyrin, a substrate of the forward reaction. When porcine meat was used, the high level of conversion of heme to Zn-protoporphyrin occurred without the addition of exogenous zinc ions, indicating that zinc ions are abundant in meat[20] and are present at sufficient levels for the conversion reaction of heme to Zn-protoporphyrin. The amount of Zn-protoporphyrin formed in meat by incubation at 4 °C was less than that by incubation at 30 °C. This result agreed with the findings that the level of Zn-protoporphyrin did not increase considerably at low temperature during the incubation and production of dry-cured ham, and it just increased during the midtemperature incubation stage of the processing.[13]

It was reported that meat extracts acted as the enzyme sources for Zn-protoporphyrin formation,[13,16,18] and the iron-removal and conversion reactions of myoglobin to Zn-protoporphyrin were successfully demonstrated.[15−17] Although exogenous protoporphyrin, myoglobin, and zinc ions were added as substrates for these reaction mixtures, the yield in the formation was low. The present data showed that not only porcine FECH in porcine muscle (raw meat) but also yeast FECH as exogenously added enzyme used the endogenous myoglobin-heme in meat as a substrate, promoting the iron-removal and conversion reactions of heme to Zn-protoporphyrin.

The oxidation of protoporphyrinogen to protoporphyrin catalyzed by protoporphyrinogen oxidase occurs in vivo in the heme-biosynthetic pathway,[31] and this protoporphyrin can be utilized for the formation of Zn-protoporphyrin. However, the sustained activity of the enzyme in meat in vitro has not been demonstrated yet, or the enzyme is unstable.[32] Because the enzyme can be destroyed easily after cell death, this oxidation process probably did not occur during the processing of dry-cured ham, indicating that the insertion of zinc into protoporphyrin after the oxidation of protoporphyrinogen cannot regularly occur. Therefore, the replacement of iron by zinc ions occurs via the reverse, and conversion reactions of heme in meat cause the formation of Zn-protoporphyrin.

The present data on the conversion reaction from heme to Zn-protoporphyrin revealed that yeast recombinant FECH can shorten the period of formation of ham pigments with a high yield. The sensory quality of dry-cured ham consists of color, flavor, and texture. Flavor involves nonvolatile (taste) and volatile compounds (aroma) including free amino acids, peptides, fatty acids, and other natural organic compounds; texture relates to myofibrillar protein breakdown, extent of drying, degradation of connective tissues, and the intramuscular fat.[3] The positive changes of flavor and texture properties can be developed up to 1−2 years of maturation and relate to the

proteolysis and lipolysis[3] in which FECH could not be involved.

The conversion of hemoporotein-heme to Zn-protoporphyrin by FECH showed an optimum at pH 6.5 (Figure 2B), whereas pH in raw meats is 5.5−6.0.[6,7] On the basis of the observations that the formation of Zn-protoporphyrin from myoglobin-heme readily proceeds in the raw tissues (Figures 5 and 6), some additional factors may be involved in the enhancement of the formation in meat. Yeast FECH showed the high conversion and iron removal activities at high NaCl concentration (up to 500 μM) (Figure 3B). This demonstrates that the enzyme can be applied to dry-cured ham production, by the addition of 20−30 g NaCl/kg raw meat,[3] in some stages to generate only the pigments, Zn-protoporphyrin, or protoporphyrin, of the ham.

Further studies will be carried out on the application of FECH to the dry-cured ham processing or that of other meat products to find suitable conditions for the enzyme reaction. Other studies should examine the effect of halophilic bacteria that can produce superior FECH or redox enzymes that are effective for the generation of ham pigments.

■ ASSOCIATED CONTENT

Supporting Information. Figures of fluorescent profiles of Zn-protoporphyrin and protoporphyrin, effect of substrate concentrations on the conversion activity, and effect of reductants on the conversion activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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INTERNAL MEDICINE

□ CASE REPORT □

False-Positive Accumulation of Metaiodobenzylguanidine in a Case with Acute Intermittent Porphyria

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Abstract

We report a 36-year-old woman presenting with hypertensive encephalopathy followed by bulbar palsy and quadriplegia. After an extensive screening for secondary causes of hypertension, the patient was suspected of having pheochromocytoma due to increased levels of catecholamines in the plasma and the urine, and positive ^{131}I-metaiodobenzylguanidine (MIBG) accumulation in the gallbladder. However, MIBG accumulation was not reproducible without any tumors accompanying this accumulation in the gallbladder. A diagnosis of acute intermittent porphyria was finally confirmed based on the characteristic pictures, increased urinary excretion of porphobilinogen, and identification of a heterozygous missense mutation of R173W in the hydroxymethylbilane synthase gene. This case highlights a pitfall in utilizing MIBG to detect a source of excessive catecholamine and also suggests the importance of having a complete clinical history and extensive work-up of any possible differential diagnosis. We also review the potential mechanism by which false-positive MIBG accumulation occurs.

Key words: acute intermittent porphyria, MIBG, pheochromocytoma, false-positive

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Introduction

Metaiodobenzylguanidine (MIBG) has been widely used as a clinical tool to detect and localize pheochromocytoma since MIBG selectively accumulates in cells derived of neuroectodermal origin, including pheochromocytoma (1). It has been shown that the specificity of MIBG accumulation, when used to localize clinical pheochromocytomas, is as high as 95-100% (2). Reflecting excessive secretion of catecholamines from tumors, clinical symptoms seen in patients with pheochromocytoma are headache, anxiety, weight loss, nausea, and paroxysmal hypertension.

Acute intermittent porphyria (AIP) is characterized by episodic acute attacks of abdominal pain, headache, paroxysmal hypertension, seizures, confusion and hallucinations. Acute porphyria attacks can be life-threatening, since the motor polyneuropathy occasionally progresses to respiratory failure requiring a mechanical ventilator. Patients suffering from AIP, however, can be totally asymptomatic during the remission periods. This is because AIP is caused by reduced enzyme activity of hydroxymethylbilane synthase involving the heme biosynthesis, and excessive accumulation of neurotoxic heme precursors is only seen during and shortly after the attacks.

As described above, there is an overlap between such clinical symptoms of AIP and those of pheochromocytoma as paroxysmal hypertension and headache, and thus patients with AIP can be misdiagnosed with pheochromocytoma or vice versa. Here, we report a case of AIP with a confusing finding of false-positive MIBG accumulation suggesting pheochromocytoma. We also review the putative mechanisms involved in the false-positive MIBG accumulation.

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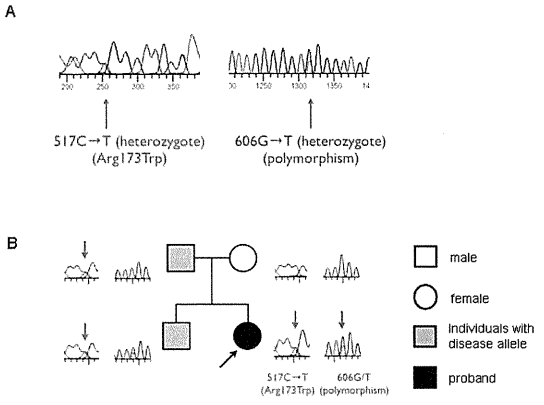

Figure 1. **Identification of a missense mutation in hydroxymethylbilane synthase in the patient and her family members. (A) Genomic DNA taken from peripheral blood mononuclear cells were PCR-amplified with specific primers for the hydroxymethylbilane synthase gene and the PCR products were directly sequenced as described previously (20). A missense mutation of R173W is indicated by a red arrow and polymorphism of 606G to T is indicated by the blue arrow. (B) Genetic testing of the family members identified the disease allele in the father and sibling of the proband. The proband is indicated by the black arrow.**

Case Report

A 36-year-old woman was admitted to the regional hospital in our area because of acute onset of visual field loss caused by hypertensive encephalopathy. While her visual field loss gradually improved by vigorous anti-hypertensive treatment, she developed progressive quadriplegia and bulbar paralysis and was therefore transferred to our university hospital. Her past medical history was unremarkable except for frequent episodes of headaches, nausea, vomiting, and generalized body pain associated with paroxysmal hypertension during the premenstrual period for more than ten years.

On physical examination, she was dehydrated and urinating port wine-colored urine. Additionally, there was apparent bulbar paralysis and quadriplegia associated with a loss of deep tendon reflexes. Since the patient was intubated and under sedation, we were not able to ask her whether she had sensation in her limbs. A blood test showed moderate anemia and the anti-ganglioside antibodies studied were negative. Cerebrospinal fluid analysis was normal. Nerve conduction study in the median, ulnar, peroneal, and tibial nerve showed normal conduction velocity with a low amplitude (amplitude and velocity was 0.79 mV and 44.0 m/sec in the median nerve and 1.5 mV and 46.4 m/sec in the ulnar) indicating axonal motor polyneuropathy while there was no involvement of sensory nerves. From the clinical picture of the patient, we strongly suspected an attack of acute porphyria. This was supported by a markedly elevated urinary concentration of porphobilinogen (134.2 mg/day; reference range less than 2 mg/day). Other urinary porphyrins were also increased; 5-aminolevulinic acid (22.3 mg/L; reference range <5 mg/L), uroporphyrin (1,820μ g/gCr; reference range <36 μg/gCr) and coproporphyrin (8,540μg/gCr; reference range 170μg/g Cr). After obtaining written informed consent from the patient and her family and approval from the ethical committee at Tottori University Hospital (Tottori, Japan), we were also able to detect a heterozygous mutation in the hydroxymethylbilane synthase gene (R 173W caused by 517C to T in exon 10), which confirmed the diagnosis of AIP (Fig. 1A). There was an additional polymorphism of 606 G to T (Fig. 1A), and genetic testing of her family members revealed that the disease allele was paternally inherited (Fig. 1B). The patient was treated with intravenous hyperalimentation, cimetidine, and chlorpromazine, and the neurological signs and symptoms gradually improved.

Before being transferred to our hospital, the patient was extensively investigated for the possibility of secondary hypertension as an etiology of hypertensive encephalopathy. Plasma levels of catecholamines and urinary levels of catecholamine metabolites were found to be elevated: plasma adrenaline (242 pg/mL; reference range <100 pg/mL), noradrenaline (2,583 pg/mL; reference range 100 to 450 pg/mL), dopamine (231 pg/mL; reference range <20 pg/mL) and urinary metanephrine (0.22 mg/day; reference range 0.04 to 0.19 mg/day), normetanephrine (1.37 mg/day; reference range 0.09 to 0.33 mg/day), VMA (9.0 mg/day; reference range 1.5 to 4.3 mg/day). Therefore, a diagnosis of pheochromocytoma was considered and ^{131}I-MIBG scintigraphy showed positive accumulation in the gallbladder (Fig. 2A, 2B). However, we were not able to find any tumorous lesions in or near the gallbladder by ultrasound (Fig. 2C). ^{123}I-MIBG accumulation studied 14 days after the ^{131}I-MIBG study showed no accumulation of MIBG in the gallbladder or adrenal gland (Fig. 2D), and there was a gradual decrease of urinary metanephrine.

Discussion

The case reported here was first presented with hypertensive encephalopathy caused by paroxysmal hypertension subsequently complicated with bulbar palsy and quadriplegia as a result of axonal motor polyneuropathy. The diagnosis of AIP was made based on the characteristic clinical presentation, increased urinary excretion of porphobilinogen, and genetic testing. It has been shown that mutations were widely distributed within the gene coding hydroxymethylbilane synthase, and the mutation identified in the present patient was also observed in Caucasians as well as in other unrelated Japanese patients with AIP (3-5). It has also been shown that the mutation is the substitution of an essential arginine to tryptophan in the active site of the enzyme, decreasing catalytic activity (<1%) (6). The patient could have been incorrectly diagnosed with pheochromocytoma based on her paroxysmal hypertension and false-positive accumulation of ^{131}I-MIBG. A misdiagnosis of this kind can easily occur in

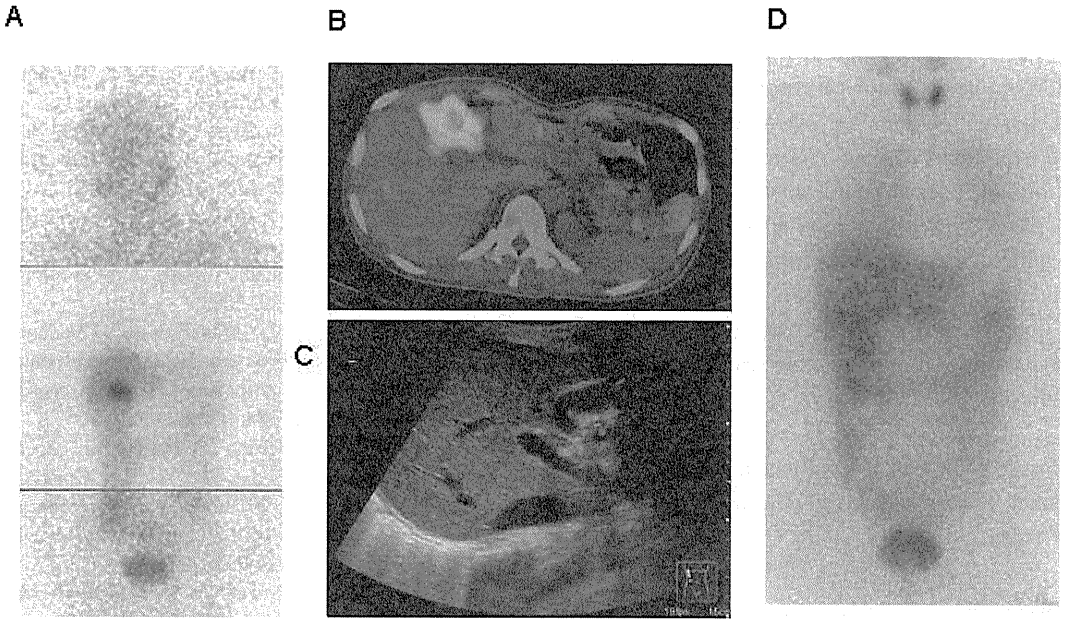

Figure 2. **False-positive accumulation of MIBG in the gallbladder. There is clear accumulation of ^{131}I-MIBG in the right upper abdomen (A), which corresponds to the gallbladder, as shown in SPECT (B). There was no detectable tumor in or near the gallbladder on ultrasound (C). ^{123}I-MIBG scintigraphy taken 14 days after ^{131}I-MIBG showed no abnormal accumulation (D).**

Japan, where AIP is extremely rare [up to 198 cases of AIP have been diagnosed as of 2009 (7)]. In addition, the clinical presentation of pheochromocytoma can vary greatly and mimic signs and symptoms seen in many other disorders (2).

It has been shown that acute porphyria attacks are commonly seen in females, although very rarely before puberty and after menopause, with a peak occurrence within the third decade (8, 9). Most patients have one or two attacks and then fully recover without a recurrence for the rest of their lives, but less than 10% develop recurrent acute attacks. During the acute attacks, reflecting augmented sympathetic activity, tachycardia, excessive sweating, and hypertension are commonly present (10). As shown herein, increased catecholamine production can be detected and may possibly suggest an incorrect diagnosis. When an acute attack of porphyria is suspected, it is essential to obtain a complete clinical history, perform an extensive work up of any possible differentiating diagnosis, and determine the urinary concentration of porphobilinogen (11).

MIBG is a norepinephrine analogue which is taken up by neuroendocrine cells through an active mechanism and stored in the neurosecretory granules. This leads to a specific concentration of the molecule in the neuroendocrine cells (1). Only limited reports of false-positive uptake in other lesions have been published. One major cause of false-positive findings is urinary tract retention, since the reagent is excreted in the urine (12). Other rare false-positive MIBG accumulations have been reported in the adrenal gland with adenoma (13), carcinoma (14) or metastatic choriocarcinoma, and adenomatous polyp of the cecum, infantile myofibromatosis (15), pancreatoblastoma (15), acute focal pyelonephritis, hepatic hemangioma, hepatocellular carcinoma (16), and juvenile capillary hemangioma (17). The non-specific accumulation of MIBG is thought to be mediated by a passive uptake and diffusion. It has been shown that non-specific uptake tends to disappear more rapidly than specific uptake (15). An augmented blood flow and enhanced diffusion of MIBG within the tumor could be putative mechanisms of false-positive MIBG uptake and accumulation in non-neuroendocrine tumors (15, 18).

There was false-positive accumulation of MIBG in the gallbladder in the case with AIP reported here. To our knowledge, there is no report of a similar false-positive MIBG accumulation. The accumulation of MIBG in the gallbladder can be considered a characteristic finding in patients with AIP. Since the liver is one of the major organs involving heme biosynthesis, which is partially defective in AIP, MIBG clearance might be disturbed during acute attacks of AIP. It is not known whether MIBG accumulates in any specific organ in patients with AIP. False-positive MIBG accumulation in the normal adrenal gland in a patient with AIP has in fact been reported (19). Thus, it seems unlikely that MIBG tends to accumulate in the gallbladder in AIP although there have been no studies confirming this. The other possibility is that the critical condition of the patient reported herein might have influenced the blood flow of the gallbladder, as the patient was severely ill and receiving intravenous hyperalimentation when ^{131}I-MIBG accumulation was studied. We were not able to find any studies on the blood flow of the gallbladder in patients with intravenous hyperalimentation, in which the gallbladder is known to dilate. However, the increased blood flow in the gallbladder would not have been the cause of false-positive MIBG accu-

mulation since the patient was still under intravenous hyperalimentation when ^{123}I-MIBG scintigraphy was studied.

In conclusion, an acute attack of porphyria should be considered when female patients of reproductive age present with characteristic episodes of neurovisceral symptoms. However, the present patient could have been incorrectly diagnosed with pheochromocytoma based on MIBG accumulation in the gallbladder. The possibility of a false-positive MIBG accumulation should always be taken into consideration when using MIBG scintigraphy to detect a catecholamine-secreting tumor, or the result of the MIBG can be misleading.

Author's disclosure of potential Conflicts of Interest (COI).
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The first two authors (T.M., and R.O.) contributed equally to this report.

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〈Regular Article〉

Skin Typing, Sun Exposure, and Sunscreen Use in a Population of Japanese Females Using an Online Interview

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Abstract

The purpose of this study was to investigate sun exposure, sunscreen use and the relationship between photoaging and sun exposure in a population of Japanese females. An online interview was performed at October 19th and 20th in 2009. Five hundred and fourteen females participated in this study. The largest number of subjects belonged to Japanese skin type class J-II (53%), J-I was second (31%), and J-III was third (16%). Fifty-seven and 45% of subjects had remarkable freckles and wrinkles, respectively. A group with much and moderate sun exposure had remarkable freckles significantly more than a group with minimum and little sun exposure ($p < 0.01$). Past history of sun exposure had no significant relation to presence of remarkable wrinkles. Twelve percent and 42% of those studied had occupational and recreational sun exposure, respectively. Eighty-one percent of those had habitual sun exposure. Sunscreens and cosmetics containing sunscreen agents were used by 86% and 65% of the subjects, respectively. Eighty-seven knew sun protection factor (SPF), but only 14% of them (12% of the total subjects) had accurate understanding the definition of SPF. Seventy-four percent knew protection grade of UVA (PA), but only 9% of them (7% of the total subjects) understood the definition of PA. This study demonstrated defects in the correct knowledge of SPF and PA and a relationship between freckles and sun-exposure history. Education in the appropriate use of sunscreens and the significance of SPF and PA is needed.

Key words: sun exposure, sunscreen use, photoaging, skin phototype.

1. Introduction

Ultraviolet (UV) light from sun exposure induces various harmful effects, *e.g.* sunburn, suntan, photoaging, and cancers on the skin. In order to prevent these harmful effects, daily protection against UV is recommended. Therefore, it is important especially for children and adults to know UV's effects on the skin. Recently most sunscreens provide broad-spectrum UV-protection. Sun protection factor (SPF) implies the ability of UVB protection of sunscreens. For UVA, Japan Cosmetic Industrial Association proposed a labeling system of protection grade of UVA (PA) using immediate tanning as a measure.[1] The European Commission has also recommended the *in vivo* persistent pigment darkening (PPD) method.[2] In 2007, Food and Drug Administration has proposed a 4-star grading of UVA protection.[3] For appropriate use of sunscreens, correct knowledge of SPF and PA is needed.

The first aim of this study was to reveal the relationship between past history of sun exposure and photoaging signs. Then we have investigated recent trends of sun exposure, freckles and wrinkles as photoaging symptoms, and skin phototype in a population of Japanese females. Previous studies[4, 5] in Japan indicated trends of lack in knowledge of SPF and PA. The second aim was to elucidate the present condition of sunscreen use and knowledge for SPF and PA.

2. Subjects and Methods

The study was carried out at October 19th and 20th in 2009. Japanese females, aged 20 to 69 years, were asked to participate. They were given an online questionnaire consisting of multiple-choice and fill-in questions. By obtaining the history of each person's cutaneous response to first sun exposure, about 1 hr at the beginning of the summer, skin phototype was determined by the following Japanese skin type (JST) classification[4]: J-I burn easily and tan minimally; J-II burn moderately and tan moderately; and J-III burn slightly and tan markedly. A modified questionnaire based on that of Kawada[4, 5] was designed to assess patterns of sun exposure, as well as patients' knowledge and use of sunscreens.

To investigate the symptoms of photoaging skin, subjects were asked whether they had remarkable signs of freckles and wrinkles that were defined as two or more freckles and wrinkles on the face. Past history of sun exposure and smoking were asked. Subjects were asked whether they had

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had much, moderate, minimum, or little sun exposure. Subjects were asked whether they had had smoking for 10 and more than 10 years, less than 10 years, or never.

For sun exposure patterns, subjects were asked about the condition of sun exposure. Occupational sun exposure was defined as daily sun exposure at outdoor labor. Recreational sun exposure was defined as not daily, but incidental sun exposure, that included swimming or climbing at resorts on holidays each average summer. Habitual sun exposure was defined as daily sun exposure, such as shopping, jogging, or walking.

To investigate the condition of usage of sunscreen products, subjects were asked if they had used sunscreens and cosmetics containing sunscreen agents. For SPF and PA, firstly subjects were asked whether they knew the term of SPF and PA. Then the subjects who answered "yes" were asked the meaning of SPF and PA with choosing multiple answers.

The results were analyzed by Fisher's exact test for independent samples.

3. Results

3–1. Demographics

Five hundred and fourteen females, aged 20 to 69 years (mean 44 years), were classified into three skin phototypes: J-I, 161 (31%); J-II, 273 subjects (53%); and J-III, 80 (16%) subjects (Table 1).

3–2. Frequency of freckles and wrinkles

For the symptoms of photoaging skin, subjects were asked whether they had remarkable signs of freckles and wrinkles. Two hundred and ninety-four (57%) subjects had remarkable freckles (Table 1). The frequent sites of freckles were cheeks (63%), vicinities of the eye (22%), arms and hands (5%), and nose (3%). Two hundred and thirty-one (45%) subjects had remarkable wrinkles (Table 1). The frequent sites of wrinkles were vicinities of the mouse (33%), vicinities of the eye (28%), forehead (27%), neck (7%), cheeks (3%), and nose (2%).

Table 1. Study population by skin phototype and presence of remarkable freckles and wrinkles.

	Total	J-I (%)	J-II (%)	J-III (%)
Population	514	161 (31)	273 (53)	80 (16)
Freckles				
Yes	294	95 (32)	152 (52)	47 (16)
No	220	66 (30)	121 (55)	33 (15)
Wrinkles				
Yes	231	73 (32)	122 (52)	36 (16)
No	283	88 (31)	151 (53)	44 (16)

3–3. Past history of sun exposure and smoking

Subjects who had had much, moderate, minimum, and little sun exposure were 23 (5%), 414 (80%), 63 (12%), and 14 (3%), respectively. A group with much and moderate sun exposure had remarkable freckles significantly more than a group with minimum and little sun exposure ($p<0.01$) (Table 2). Subjects who had had smoking for 10 and more than 10 years, less than 10 years, and never were 63 (12%), 72 (14%), and 379 (74%), respectively. There was no significant difference about remarkable wrinkles between a group with much and moderate sun exposure and a group with minimum and little sun exposure. On the other hand, a group with smoking history of 10 and more than 10 years had remarkable wrinkles significantly more than a group with less than 10 years ($p<0.01$) and a group without smoking history ($p<0.05$) (Table 2).

3–4. Sun exposure patterns

Subjects were asked about the condition of sun exposure. Sixty (12%) of the subjects experienced occupational sun exposure. Two hundred and seventeen (42%) of the subjects experienced occasional sun exposure. Four hundred and fourteen (81%) of the subjects experienced habitual sun exposure. Of the total subject population, 461 (90%) experienced sun exposure and 53 (10%) had neither.

3–5. Sunscreen use

Subjects were asked if they used sunscreens and cosmetics containing sunscreen agents. Four hundred forty-two (86%) and 335 (65%) used sunscreens and cosmetics containing sunscreen agents, respectively (Table 3). Other methods for sun protection were asked. Two hundred and eighty-seven (56%) used hats, 284 (55%) did umbrellas, 183 (36%) did shirts with long sleeves, 140 (27%) did gloves, and 120 (23%) did sunglasses (Table 3).

Table 2. Relationship of sun exposure and tobacco smoking with presence of remarkable freckles and wrinkles.

	Freckles		Wrinkles	
	Yes (%)	No (%)	Yes (%)	No (%)
Sun exposure				
Much and Moderate	262 (60)*	175 (40)	202 (46)	235 (54)
Minimum and Little	32 (42)	45 (58)	29 (38)	48 (62)
Smoking				
10 and More than 10 years	58 (92)	5 (8)	37 (59)**, ***	26 (41)
Less than 10 years	60 (83)	12 (17)	23 (32)	49 (68)
None	332 (88)	47 (12)	171 (45)	208 (59)

*$p<0.01$ compared with a group with minimum and little sun exposure in Fisher's exact test.
**$p<0.01$ compared with a group with smoking history of less than 10 years in Fisher's exact test.
***$p<0.05$ compared with a group with no smoking history in Fisher's eact test.

Table 3. Methods for sun protection.

	Total (%)
Sunscreens	442 (86)
Cosmetics containing sunscreen agents	335 (65)
Hats	287 (56)
Umbrellas	284 (55)
Shirts with long sleeves	183 (36)
Gloves	140 (27)
Sunglasses	120 (23)

Table 4. Reasons for using sun products.

	Total (%)
Prevent sunburn	325 (63)
Prevent suntan	266 (52)
Prevent freckles and wrinkles	457 (89)
Prevent skin cancer	104 (20)
Promote tanning	14 (3)

Table 5. Presence of correct knowledge about the definition of sun protection factor (SPF) and protection grae of UVA (PA).

	Total (%)	J-I (%)	J-II (%)	J-III (%)
SPF				
knows SPF	449 (87)	135 (84)	248 (91)	66 (82)
(correctly knows)	(62 (12))	(23 (14))	(32 (12))	(7 (9))
not know SPF	65 (13)	26 (16)	25 (9)	14 (18)
PA				
knows PA	378 (74)	123 (76)	199 (73)	56 (70)
(correctly knows)	(34 (7))	(13 (8))	(17 (6))	(4 (5))
not know PA	136 (26)	38 (24)	74 (27)	24 (30)

Users of sunscreen products were asked to select reasons for using sunscreen. Three hundred and twenty-five (63%) used them to prevent sunburn, 266 (52%) to prevent suntan, 457 (89%) to prevent freckles and wrinkles, 104 (20%) to prevent skin cancer, and 14 (3%) to promote tanning (Table 4).

3–6. Knowledge about the definition of SPF and PA

When asked about the SPF of sunscreens, 449 (87%) of all the subjects answered they knew the term of SPF (Table 5). Of these 449 subjects, 62 (14% of those; 12% of all subjects) answered correctly that the higher the SPF, the more protection the sunscreen provides (Table 5). More J-I (14%) and J-II subjects (12%) correctly knew SPF than J-III (9%) without statistical significance. Remaining subjects said higher SPF indicates less protection, or did not know the definition of SPF.

When asked about the PA of sunscreens, 378 (74%) of all the subjects answered they knew the term of PA (Table 5). More J-I subjects (76%) knew PA than J-III (70%). Thirty-four (9% of those; 7% of total) of these 378 subjects answered correctly that the higher the PA, the more UVA protection the sunscreen provides (Table 5). Remaining subjects misunderstood it, or had no idea. More J-I (8%) and J-II subjects (6%) correctly knew PA than J-III (5%) without statistical significance.

4. Discussion

Photoaging skin is characterized with freckles, wrinkles, rough skin, and premalignant and malignant cutaneous tumors. We have investigated the relationship of freckles and wrinkles with past history of sun exposure. A group with much and moderate sun exposure had remarkable freckles significantly more than a group with minimum and little sun exposure. Presence of remarkable wrinkles was not related to sun-exposure history, but to smoking history with a statistical significance in our study. In a population of Japanese, the number of wrinkles was significantly related to total hours spent outside in life.[6] Tobacco-smoke is known to be involved in formation of wrinkles.[7] The other study reported that sun exposure and smoking independently contributed to facial wrinkles.[8] This discrepancy may be caused by the differences of the population studied. Our population composed of only females, whereas other studies included males and females. Then, wrinkles in our population may be influenced by smoking rather than sun exposure.

More subjects experienced habitual sun exposure (81%) than occupational (12%) and recreational (42%) sun exposure. These trends in sun exposure were different from the previous studies.[4, 5] that demonstrated more subjects spent recreational sun exposure. More subjects (86%[4] and 88%[5]) had spent recreational sun exposure than habitual sun exposure (23%[4] and 26%[5]). Recent tendency implies that more Japanese female people may prefer habitual sun exposure. Therefore, more appropriate information about sun protection methods for habitual sun exposure should be provided.

Eighty-six percent and 65% used sunscreens and cosmetics containing sunscreen agents, respectively. This implies that Japanese females use both of sunscreens and UV-protective cosmetics. For other methods of sun protection, more than half of the subjects studied used hats and umbrella that was similar in USA[9] and Greece.[10] Twenty-three percent in our study used sunglasses, whereas 57% in USA and 76% in Greece used them. This difference may be caused by the cultural difference. In Japan, it is necessary to advise the use of other methods of sun protection as well as sunscreens.

Ninety-seven percent of all the subjects used sunscreens to protect from acute and/or chronic effects. Sunscreens were used to promote tanning only in 3% that was much lower than 11%[4] and 10%[5] in the previous studies. In Japanese females, the appropriate use of sunscreens has become widespread.

Eighty-seven percent of all subjects knew the term of SPF, while only 14% of those (12% of all subjects) had an accurate understanding the definition of SPF. This figure was similar to previous studies (10～13%)[4, 5]. Most subjects (84%) knew the term of PA, whereas only 9% of those (7% of all subjects) understood the correct meaning of PA. Previous study in 2002[5] indicated that most subjects (93%) did not know the term of PA. These results suggested that education in the appropriate use of

sunscreens and the meaning of SPF and PA is needed. Dermatologists and manufacturers should make more efforts to educate consumers about sunscreens.

In conclusion, our study revealed that past history of sun exposure had significant relation to presence of remarkable freckles, but not to wrinkles. Our study also demonstrated present condition of sunscreen use and lack of correct knowledge of SPF and PA. Further plan to increase the use and knowledge of sunscreens is currently under way.

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Review Article

Protection and Therapy of Photoaging

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Abstract

Chronic and repeated sun exposure causes photoaging skin that includes solar lentigines, wrinkles, changes of texture, benign tumors, and cutaneous cancers. Various symptoms of photoaging have been a great concern in dermatology. Photoprotection using sunscreens is recommended to prevent these signs. Two measures, sun protection factor (SPF) for UVB and protection grade of UVA (PA) for UVA, are described on the label of sunscreens. Our recent investigation revealed defects in the correct knowledge of SPF and PA and a relationship between freckles and sun-exposure history. Education in the appropriate use of sunscreens and the significance of SPF and PA is needed.

Solar lentigines on the face decrease quality of life. Previous laser therapies cause erosion and crusts with downtime for the treatment of pigmentary lesions. Then, intense pulsed light (IPL) sources have been developed as noninvasive and nonablative modalities for facial solar lentigines. We demonstrated clinical effectiveness of an IPL source for solar lentigines and ephelides on the face with well tolerability. Then, we performed a histopathological study that indicated IPL produced highly selective photothemolysis of melanin pigment in the lesions of solar lentigines, leading to the clinical improvement. Moreover, we showed clinical effects of a novel IPL source on solar lentigines and ephelides. In the future, phototherapy including IPL sources will develop with more effectiveness and safety.

KEY WORDS: photoaging, photoprotection, sunscreen, solar lentigo, therapy, intense pulsed light source

Introduction

Ultraviolet (UV) light from sun exposure induces various harmful effects, *e.g.* sunburn, suntan, photoaging, and cancers on the skin. Chronic exposure of UV to the skin causes photoaging. Photoaging skin is characterized with sallowness, mottled pigmentation, solar lentigines, dry and rough skin, loss of skin tone, leathery texture, laxity, coarse and fine wrinkles, and benign and malignant tumors [1].

In order to prevent the various signs of photoaging, daily protection against UV is recommended. Therefore, it is important for children and adults to know the appropriate methods of photoprotection including sunscreens.

Topical agents such as glycolic acid, retinoids, ascorbic acid, a variety of chemical peeling agents, dermabrasion, epidermabrasion, and laser skin resurfacing have been reported for the treatment of the symptoms of photoaging [2]. These effective therapies, mostly invasive, usually need patient downtime and sometimes cause adverse effects. Noninvasive and nonablative treatments without patient downtime are required. Then, intense pulsed light (IPL) therapy was developed.

In this review article, trends of sun protection and IPL therapy for solar lentigines from our investigation have been demonstrated.

Protection

Recently most sunscreens provide broad-spectrum UV-protection. Sun protection factor (SPF) implies the ability of UVB protection of sunscreens. For UVA, Japan Cosmetic Industrial Association proposed a labeling system of protection grade of UVA (PA) using immediate tanning as a measure [3]. The European Commission has also recommended the *in vivo* persistent pigment darkening (PPD) method [4]. In 2007, Food and Drug Administration has proposed a 4-star grading of UVA protection [5]. For appropriate use of sunscreens, correct knowledge of SPF and PA is needed.

Kawada *et al.*[6] studied the relationship between past history of sun exposure and photoaging signs. They investigated recent trends of sun exposure, freckles and wrinkles as photoaging symptoms, and skin phototype in a population of Japanese females. Then, they investigated the condition of sunscreen use and knowledge for SPF and PA in 2009. Five hundred and fourteen females, aged 20 to 69 years (mean 44 years), Japanese females, participated. They were given an online questionnaire consisting of multiple-choice and fill-in questions. By obtaining the history of each person's cutaneous response to first sun exposure, about 1 hr at the beginning of the summer, skin phototype was determined by the following Japanese skin type (JST) classification [4]: J-I burn easily and tan minimally; J-II

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burn moderately and tan moderately; and J-III burn slightly and tan markedly. Subjects were classified into three skin phototypes: J-I, 161 (31%); J-II, 273 subjects (53%); and J-III, 80 (16%) subjects. Subjects who had had much, moderate, minimum, and little sun exposure were 23 (5%), 414 (80%), 63 (12%), and 14 (3%), respectively. A group with much and moderate sun exposure had remarkable freckles significantly more than a group with minimum and little sun exposure ($p < 0.01$). Eighty-seven percent of all subjects knew the term of SPF, while only 14% of those (12% of all subjects) had an accurate understanding the definition of SPF (***Table 1***). This figure was similar to previous studies (10-13%)[7,8]. Most subjects (84%) knew the term of PA, whereas only 9% of those (7% of all subjects) understood the correct meaning of PA (***Table 1***). Previous study in 2002[8] indicated that most subjects (93%) did not know the term of PA. These results suggested that education in the appropriate use of sunscreens and the meaning of SPF and PA is still needed. Dermatologists and manufacturers should make more efforts to give consumers more information about sunscreens.

Table 1. Presence of correct knowledge about the definition of sun protection factor (SPF) and protection grade of UVA (PA)

	Total (%)	J-I (%)	J-II (%)	J-III (%)
SPF				
knows SPF	449 (87)	135 (84)	248 (91)	66 (82)
(correctly knows)	(62 (12))	(23 (14))	(32 (12))	(7 (9))
not know SPF	65 (13)	26 (16)	25 (9)	14 (18)
PA				
knows PA	378 (74)	123 (76)	199 (73)	56 (70)
(correctly knows)	(34 (7))	(13 (8))	(17 (6))	(4 (5))
not know PA	136 (26)	38 (24)	74 (27)	24 (30)

Therapy of photoaging

IPL, a broadband visible light emitted from a noncoherent, nonlaser, filtered flashlamp, has been developed as a new noninvasive method[2,9,10]. IPL is effective for superficial rhytides, wrinkling, skin coarseness, irregular pigmentation, pore size, and telangiectases[2,9].

1. IPL therapy for solar lentigines and ephelides

We performed an open study of IPL for the treatment of solar lentigines and ephelides[11]. Sixty patients (56 women, 4 men), age 20–82 years (mean 50 years), with facial pigmentary lesions participated in this study. Facial pigmentary lesions were clinically diagnosed as solar lentigines, solar lentigines + ephelides, and ephelides. Solar lentigines were also classified into small (1 cm and less than 1 cm) and large plaques (more than 1 cm). A noncoherent, filtered, broadband, pulsed flashlamp (NatuLight, Lumenis CO., LTD. (Koto-ku, Tokyo Japan)) emitting in the range of 500–1200 nm was used for all treatments. Each patient received three to five treatments (average number 4.0), given at 2- to 3-week intervals. Treatment fluences ranged from 20 to 24 J/cm^2. Energy was delivered in double- or triple-pulse trains of 2.6–5.0 msec with pulse delays of 20 msec. Cutoff filters of 560 nm were used. Overall, 48% of subjects showed more than 50% improvement and 20% had more than 75% improvement. Only one case had erosions, and no other cases showed hyperpigmentation, scarring, or downtime. In the solar lentigines group, 40% of subjects showed more than 50% improvement and 16% had more than 75% improvement. IPL was effective for small plaques of solar lentigines, with 48% having more than 50% improvement. On the contrary, 72% of the patients with small + large and large plaques had poor or slight improvement. The solar lentigines + ephelides and ephelides groups showed great improvement, with 75% and 71%, respectively, having more than 50% improvement. Representative cases with marked response are shown in ***Fig. 1*** and ***Fig. 2***. Therefore IPL may be added to the panel of modalities used for the treatment of ephelides and small-type solar lentigines.

Asian skin easily associates with hyperpigmentation after various therapies for photoaging skin, such as laser surgery[12,13], chemical peeling, and CO_2 laser resurfacing. Q-switched ruby laser (QSRL) therapy, effective for solar lentigines in Japanese patients, causes postinflammatory hyperpigmentation, especially in patients with J-III[14]. QSRL therapy should be performed carefully because of hyperpigmentation in J-III subjects with more melanogenicity. However, IPL therapy in our study showed no postinflammatory pigmentation in any subjects including J-III patients, indicating that IPL may have an advantage over QSRL for the treatment of pigmentary disorders. Only one patient in our study showed burn from IPL. This patient's severe response may have been induced by ample amounts of melanin pigment in the lesion which is a target chromophore of IPL. Darker lesions and complexion should be treated cautiously because unexpected response may occur during IPL therapy. IPL therapy proved to be effective and tolerable for the patients, suggesting that IPL may be a possible good modality for solar lentigines and ephelides.

2. The mechanism of IPL therapy for solar lentigines

In order to reveal the mechanism of efficacy of intense pulsed light for solar lentigines, we performed histopathological examination[15]. Twenty patients (18 females and 2 males), ages 30–78 years (mean 52 years), with solar lentigines participated in this study. Sequential histological pictures of small solar lentigines showed subepidermal cleft, vacuolization of pigmented basal keratinocytes and melanocytes, the disappearance of pigmentary incontinence in the papillary dermis at 30 m, lymphocytic infiltration in the upper dermis at 6 h, degenerated epidermis and enlargement of the cleft at 24 h, and crust at 7 days after irradiation (***Fig. 3***). Results with Masson-Fontana staining also revealed vacuolated change of basal keratinocytes and melanocytes, degenerated epidermis with melanin pigments, and crust formation containing ample melanins with decrease in melanin of basal cells. We demonstrated that clinical tiny-crust in the lesions of solar lentigines was the consequence of micro-crust formation histopathologically. Crust-formation was localized on the part of pigment spots, indicating the specificity of IPL for epidermal melanin under our condition. Formation and drop-off of the crusts lead to clinical improvement of pigmentary lesions of solar lentigines. Transient inflammation with redness was seen, while no adverse sequelae such as hyperpigmenation and scarring appeared. Therefore, IPL may be a modality for solar lentigines as a highly selective therapy for pigment removal.

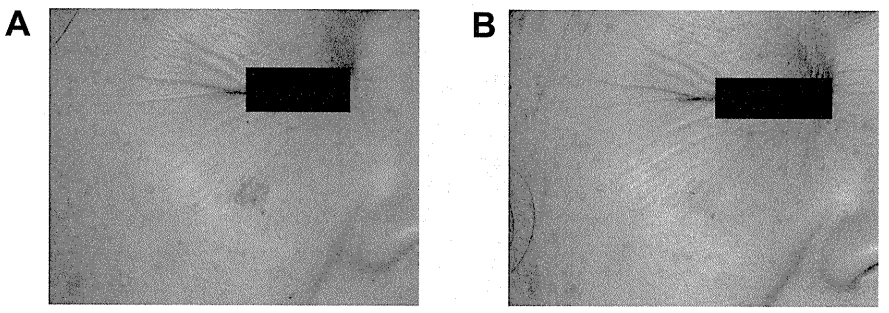

Fig. 1. **A 51-year-old female**
A) before and B) 2 weeks after five IPL treatments.
Large and small pigmented lesions improved.

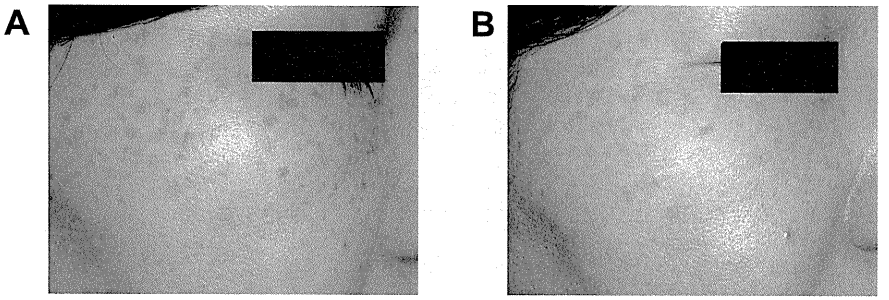

Fig. 2. **A 45-year-old female**
A) before and B) 2 weeks after five IPL treatments.
Small pigmented lesions improved.

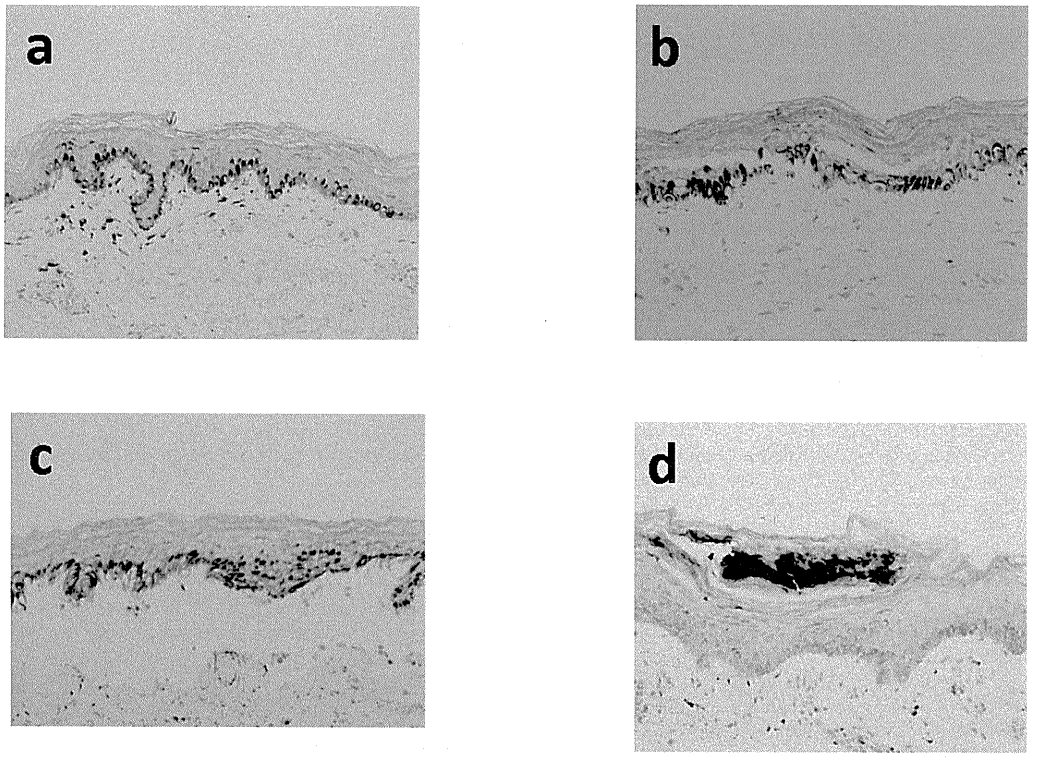

Fig. 3. **Sequential histopathological findings of pigment spots of solar lentigines.**
Pigment spots at pre-irradiation (a), 30 min (b), 24 h (c), and 7 days (d)
after irradiation of intense pulsed light were shown (a-d, hematoxylin-eosin staining; x 200).

3. Novel IPL modality for solar lentigines and ephelides

Recently a novel IPL source (Lumenis One™, Lumenis CO., LTD.) with stronger irradiation and various filters has been developed as a second generation of IPL. Lumenis One is a phototherapy unit that composes of IPL, LightSheer diode laser for hair removal, and Multi-Spot Nd:YAG laser for leg veins and deeper vascular lesions. IPL of Lumenis One is characterized with stronger intensity, two spot sizes, replaceable seven filters, and an integrated dynamic cooling device, designating as a second platform of IPL. We investigated clinical effectiveness of Lumenis One on facial pigmentary lesions [16]. Eighteen Japanese female patients aged 22–72 years (mean 50 years), with facial pigmentary lesions (solar lentigines, solar lentigines + ephelides, and ephelides), participated in this study. Each patient received three to five treatments. Each treatment, given at 2–3-week intervals, was administered on the face. Treatment fluences ranged from 12 to 14 J/cm^2. Energy was delivered in double pulse trains of 4.0 ms with pulsedelays of 20 ms. Cut-off fillters of 560 nm were used. All the patients completed the study. No adverse effectswere seen in any patients. Physicians' overall assessments demonstrated clinical improvement in the total population, indicating that 28% of patients showed marked improvement, none did moderate improvement, and 39% did slight improvement. Thirty-three percent showed no change, while no patients did "worsened". The melanin index decreased after the treatment comparing before the treatment. These results revealed the clinical effectiveness with well tolerability on facial pigmentary lesions, such as solar lentigines, ephelides, and solar lentigines + ephelides.

Conclusion

Photoaging skin is an important issue in the dermatological field. Various signs of photoaging include pigmentary lesions, wrinkles, changes of texture, benign tumors, and skin cancers. Daily sun protection is important to prevent these symptoms. Recent development of phototherapy helps improvement of pigmentary lesions.

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—使用試験—

新規紫外線吸収剤を配合したブロードスペクトラム日やけ止め化粧料 BSUS ミルク-1 の光線過敏症患者への使用試験

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4種の紫外線吸収剤と散乱剤を配合することで UV-A の紫外線防御効果を強化し，UV-A，B の両波長領域にわたって高い紫外線防御能を有するブロードスペクトラム日焼け止め化粧料 BSUS ミルク-1 について，光線過敏症患者39例に対する臨床試験を6大学にて実施し，有用性の評価を行った。6～13週間の使用試験の結果，評価対象例38症例中31例（81.6%）において，使用感，安全性に問題がなく，十分な紫外線防御効果が認められ，本試験品が有用であることが示された。また，今回の結果においては，作用波長に UV-A を含む患者群の症状改善傾向が強く，本試験品がこれら患者群に対してより有効であったことを示していた。
（皮膚の科学，*10: 424-441, 2011*）

キーワード：日焼け止め化粧料，光線過敏症，紫外線防御，紫外線吸収剤，UV-A

はじめに

慢性光線性皮膚炎（chronic actinic dermatosis : CAD）や薬剤性光線過敏症などの光線過敏症患者においては症状の発現と増悪の予防のために日常生活での遮光が必要不可欠である。これら疾患の中でも CAD 等の内因性光線過敏症についてはその発症機序が明確になっていないものも多く，根本治療の有効な手段はいまだ見出せていないのが現状である。そのため，このような内因性疾患患者においては日常生活での永続的な遮光が必須であり，疾患が患者の QOL に多大な影響を及ぼしてくる。

日常生活における光線曝露防止のための具体的手段としては，日中の外出制限や日傘，帽子，衣類，日焼け止め化粧料などの使用による光防御が挙げられる[1)]。その中で光線過敏症患者に対して日焼け止め料の使用は非常に有効であることが多くの臨床試験により実証されている[2-8)]。しかしながら，塗布後の白さや圧迫感，塗布行為の煩わしさなど，使用感に関する問題が日焼け止め料の適切な使用を拒む原因となっていることが多い。特に CAD 患者は高齢男性に多いため，化粧料の使用経験に乏しく，日焼け止め化粧料の使用に対して強い抵抗があると考えられる。

日焼け止め料の光防御効果は日焼け止め料に含まれる紫外線散乱剤や紫外線吸収剤によるものであるが，これらの成分はそれぞれの成分固有の紫外線散乱あるいは紫外線吸収スペクトル特性を有しており，防御できる光線波長領域や光線強度に違いがある。

酸化亜鉛などの紫外線散乱剤は可視光領域も含めた比較的幅広い領域の波長に対して紫外線散乱作用を有しているものが多い。しかし，使用時に塗布部位が白くなりやすく，肌表面での物理的な閉塞作用から塗布

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していることを負担に感じやすいといった傾向がある。

一方で，メトキシケイヒ酸エチルヘキシルなどに代表される紫外線吸収剤は狭い紫外線波長領域，特にUV-B領域に対する吸収スペクトルを有しているものが多く，単独では幅広い波長領域の光線に対して高い防御力を発揮することが困難である[9,10]。しかしながら近年，幅広い紫外線波長領域に対する吸収スペクトルを有する紫外線吸収剤や，これまで汎用されてきた紫外線吸収剤とは異なるUV-A領域に対する吸収スペクトルを持つ紫外線吸収剤など[11]，新たなタイプの吸収剤の化粧品における使用が厚生労働省から認可され[12~15]，これらを組み合わせて配合し広範な紫外線波長領域にわたって高い防御効果のある日焼け止め料が市場に登場してきている。

日焼け止め料についてはこれまでにも数多くの臨床試験が実施されており[2~8,18,19]，被験者としてアトピー性皮膚炎患者[5~8,18]，あるいは健常小児を対象としている試験[19]などにおいては，試験品に紫外線吸収剤無配合の日焼け止め料を用いているものがほとんどである。また，光線過敏症患者を対象にした臨床試験でも同様に紫外線吸収剤無配合の日焼け止め料を用いた報告が多く[2~8]，紫外線吸収剤配合タイプの日焼け止め料を用いた臨床試験は市橋らの使用試験報告[4]などがあるものの，数としては多くはない。

今回，我々は紫外線散乱剤と4種の紫外線吸収剤を組み合わせUV-A，Bの両波長領域にわたって高い紫外線防御能を有するという特徴を持ったブロードスペクトラム日焼け止め化粧料を用いて光線過敏症患者を対象にした臨床試験を実施したので報告する。

試　験　方　法

1．対　象

平成21年7月から平成22年4月までに6大学病院皮膚科を受診し，日常の生活指導において日焼け止め料の使用が望ましいと考えられるCAD，多形日光疹(polymorphous light eruption：PLE)，日光蕁麻疹，外因性光線過敏症などの光線過敏症患者で，作用波長がUV-Aかつ/またはUV-Bで，文書による試験参加の同意が得られた者を対象にした。なお，遺伝性の光線過敏症患者は今回の被験対象から除外した。また下記の項目に該当する患者も除外した。

①被験部位に湿潤傾向の強い急性病変のある患者

②試験途中で治療方法あるいは投与薬剤を変更する必要が予想されている患者

③本試験の評価に影響を及ぼすと考えられる合併症のある患者

④その他，担当医師が不適当と判断した患者

Table 1　試験実施施設

施設名	責任医師および担当医師
京都大学医学部付属病院皮膚科	宮地良樹*，加藤真弓**（現：高槻赤十字病院皮膚科）
神戸大学医学部付属病院皮膚科	錦織千佳子
関西医科大学付属病院皮膚科	岡本祐之，水野可魚，野田佳織，山崎文和
大阪医科大学付属病院皮膚科	森脇真一
近畿大学医学部付属病院皮膚科	川田暁，川原繁（現：金沢赤十字病院皮膚科），大磯直毅，吉永英司，東森倫子
和歌山県立医科大学付属病院皮膚科	古川福実

* 主任研究者　** 論文執筆者

2．患者の同意

本試験の実施については各試験実施施設における倫理委員会で審査ならびに承認を受けた。本試験への参加についての患者の同意に関しては，本使用試験の目的，内容，試験参加に同意しない場合であっても不利益を受けないこと，試験に同意した後でも患者は随時同意を撤回できること，試験参加者の個人情報の管理方法などを患者に説明した後に，文書によって承諾を得た。なお，患者が未成年の場合には患者ならびにその保護者からの同意を得た。

3．試験実施期間および実施施設

平成21年7月から平成22年6月にかけて，以下に示す6大学病院皮膚科にて実施した（**Table 1**）。

4．試験品

本試験に用いた試料（BSUSミルク-1）は株式会社コーセーにより開発された日焼け止め化粧料で，紫外線散乱剤として酸化亜鉛を，紫外線吸収剤として，メチレンビスベンゾトリアゾリルテトラメチルブチルフェノールおよびビスエチルヘキシルオキシフェノールメトキシフェニルトリアジン，メトキシケイヒ酸エチルヘキシル，ジエチルアミノヒドロキシベンゾイル安息香酸ヘキシルを配合し，UV-BからUV-Aにわたる広い波長領域を防御する。特に市販の一般的な日焼け止め化粧料に比べてUV-A領域に対して優れた紫外線防御能を持つように設計されている。また，無香料，無着色でパラベンを配合していない。

本試験品の遮光効果は，UV-Bについては実測値SPF53.1，UV-Aについては実測値PFA 17.6であり，これは日本化粧品工業会のSPF測定法基準[16]およびUV-A防止効果測定法基準[17]に則り測定している。

試験品の配合成分は**Table 2**に示してある。

5．使用方法

被験者に外出前，顔面および前腕を含む露光部全体に試験品を適量（2 mg/cm^2）塗布するように指導し

Table 2　配合成分

原料分類	成分名
紫外線吸収剤	メチレンビスベンゾトリアゾリルテトラメチルブチルフェノール，ビスエチルヘキシルオキシフェノールメトキシフェニルトリアジン，メトキシケイヒ酸ヘチルヘキシル，ジエチルアミノヒドロキシベンゾイル安息香酸ヘキシル
紫外線散乱剤	酸化亜鉛
乳化剤	ステアロイルメチルタウリンNa，ステアリン酸グリセリル，PEG-40水添ヒマシ油，ラウリン酸ポリグリセリル，PEG-10水添ヒマシ油，ポリソルベート80，トリセテアレス-4リン酸，オレイン酸ソルビタン
油　剤	シクロメチコン，ジメチコン，フェニルトリメチコン，イソノナン酸イソトリデシル，イソヘキサデカン
増粘剤	(アクリル酸/アクリロイルジメチルタウリンNa)コポリマー，ヒドロキシプロピルメチルセルロース
基　剤	シリカ，セテアリルアルコール，ミリスチン酸
保湿剤	BG
防腐剤	フェノキシエタノール
酸化防止剤	BHT
溶　媒	水

た。具体的には「顔に塗布する場合には，0.3g（小指の先大）を掌にとり，一度丁寧に顔全体に伸ばす。伸ばしきったところで同じ操作を繰り返して重ね塗りを行う。頸部および手背部に塗布する場合には，それぞれ0.3g（小指の先大）を掌にとり，丁寧に塗布部全体に伸ばす。伸ばしきったところで同じ操作を繰り返して重ね塗りを行う」と指導した。なお，長時間の外出時には2時間ごとに試験品を塗り直し，手を洗ったり汗をかいたりタオルで汗を拭ったりした場合にも必ず塗り直しを行うように指導した。

治療中の被験者に対しては試験期間中も従来行ってきた治療方法を継続して行うこととし，試験期間中に治療方法や投与薬物等の変更があった場合はケースカードに記録した。また，試験期間中は他の日焼け止め料の使用は禁止した。ただし，日焼け止め料以外の化粧品に関しては，試験開始前までに使用しており安全性が確認されているもののみ使用を認め，試験期間中に新たな製品を使用することは禁止した。

6．試験期間

原則として10週間とし，6週間以上使用した症例を試験完遂症例として取り扱った。また，最長3ヶ月までの継続使用を認めた。

7．観察日

観察は試験開始時，ならびに試験開始2～4週間後，6週間後，試験終了時に実施することにした。途中経過観察については，被験者の来院スケジュール等に合わせて前後1週間程度の観察日のずれ，さらに，やむを得ない場合には観察の省略を許容した。副作用が認められた患者から中止希望の申し出があった等の理由で，試験を中止した場合は，中止した年月日およびその理由をケースカードに記入した。

8．調査・観察項目

1）被験者背景情報

被験者イニシャル，性別，生年月日，年齢，試験品No.，カルテNo.，施設名，担当医師，患者同意年月日，対象疾患名とその症状および重症度，罹病期間，作用波長，MED，MRD，対象疾患に対する治療薬，合併症，評価部位の皮膚所見，試験開始前の日焼け止め料の使用の有無，同意書の有無をケースカードに記入した。

2）使用状況

試験開始時を除く各観察時に試験品の使用状況を問診し，使用頻度を以下の5段階で記録した。①毎日使用，②週に4～6日使用，③週に1～3日使用，④使用せず，⑤その他（用量・用法の逸脱など）

また，紫外線曝露時の試験品の使用状況についても，以下の5段階で記録した。①毎回，適切に使用，②ほぼ適切に使用，③外出時，使用しないことも度々あった，④使用せず，⑤紫外線曝露なし

3）皮膚所見

各観察時に被験部位における皮膚の状態について肉眼観察を行い，項目（乾燥，落屑，紅斑，浮腫，丘疹）ごとにその程度を下記5段階で記録した。①なし（症状が見られない），②軽微（ごくわずかに症状が見られる），③軽度（少し症状が見られる），④中等度（明らかな症状が見られる），⑤重度（著しい症状が見られる）

また，初回観察時と比較した全般的改善度を下記5段階で評価した。＋＋　著しく改善，＋　改善，±　やや改善，－　不変，×　悪化

4）その他の記載事項

初回観察時より写真撮影の実施の有無について記入

Table 3　有効性の判定基準

判　定	試験開始前に日焼け止め料を使用していた被験者	試験開始前に日焼け止め料を使用していなかった被験者
①有効	症状の良い状態を維持した，症状が改善傾向になった	症状が消失または改善した
②やや有効	日焼け止め料の使用を中止するよりは良い状態を維持した	症状が一部改善または改善傾向になった
③無効	日焼け止め料未使用の場合と同等の状態に悪化した	症状に変化がなかった，症状の一部は改善したが一部は悪化した
④悪化	日焼け止め料未使用の場合の症状よりも悪化した	症状が悪化した

Table 4　症例についての詳細情報

症例No.	性別	年齢	原疾患	作用波長	MED (mJ/cm²)	MRD (J/cm²)	症状(重度)	焼け止め使用歴	試験継続日数	皮膚所見 初回診察時 乾燥	落屑	紅斑	浮腫	丘疹	最終診察時 乾燥	落屑	紅斑	浮腫	丘疹	全般的改善度(最終判定)	有害事象の発現	安全度	有効性	有用性	中止等
1	男性	84	CAD	AB	5	2.5	高度	使用中	77	3	3	3	1	1	1	1	2	1	1	著しく改善	無	安全	有効	○	
2	男性	63	外因性光線過敏症	B			高度	使用中	42	3	2	5	5	5	3	2	5	5	5	不変	無	安全	やや有効	○	
3	男性	65	CAD	AB	10	1.6	高度	使用中	15	(2)	(3)	(3)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(改善)	無	(安全)	(有効)	×	中止(使用感不良)
4	男性	12	PLE	A		12	中等度	使用中	77	1	2	3	1	4	1	1	2	1	1	改善	無	安全	有効	○	
5	女性	41	PLE	A		12	中等度	使用中	70	4	3	4	1	3	1	1	2	1	2	改善	無	安全	有効	○	
6	男性	78	CAD	AB	20	6	高度	未使用	68	3	3	4	3	4	1	1	2	1	2	改善	無	安全	有効	○	
7	男性	69	CAD	A		12	中等度	未使用	36	(2)	(1)	(1)	(1)	(2)	(2)	(1)	(2)	(1)	(1)	(改善)	有	やや問題あり	(やや有効)	×	中止(有害事象)
8	男性	80	CAD	AB	UVB 30	UVA6	高度	未使用	75	2	3	4	1	4	2	1	2	1	2	著しく改善	無	安全	有効	○	
9	女性	77	CAD	AB	10	1	高度	使用中	1	(2)	(3)	(3)	(2)	(3)	(2)	(3)	(3)	(2)	(3)	(不変)	有	問題あり	(—)	×	中止(有害事象)
10	女性	48	PLE	A	60	6	中等度	使用中	72	1	1	2	2	2	1	1	1	1	1	改善	無	安全	やや有効	○	
11	男性	74	PLE	B	50		軽度	未使用	52	2	1	2	1	2	2	1	1	1	1	改善	無	安全	有効	○	
12	男性	64	外因性光線過敏症	A		10	高度	未使用	71	1	1	3	3	3	1	1	1	1	1	著しく改善	無	安全	有効	○	
13	男性	63	CAD	B	7.5		中等度	使用中	83	1	1	2	2	3	1	1	2	2	2	やや改善	無	安全	有効	○	
14	男性	73	CAD	AB	50	10	高度	使用中	63	4	4	4	4	4	2	2	2	1	2	著しく改善	無	安全	有効	○	
15	女性	62	外因性光線過敏症，全身性エリテマトーデス	A			軽度	未使用	1	(2)	(1)	(3)	(1)	(1)	(1)	(1)	(3)	(1)	(1)	(やや改善)	有	問題あり	(有効)	×	中止(有害事象)
16	女性	71	外因性光線過敏症，尋常性白斑				中等度	未使用	1	(2)	(2)	(1)	(1)	(1)	(—)	(—)	(—)	(—)	(—)	(悪化)	有	問題あり	(悪化)	×	中止(有害事象)
17	男性	49	CAD	B	12.5		中等度	使用中	55	1	1	2	1	3	1	1	2	1	3	不変	無	安全	やや有効	○	
18	女性	71	外因性光線過敏症				軽度	未使用	74	1	1	2	1	2	1	1	1	1	1	改善	無	安全	有効	○	
19	男性	53	CAD	AB	50	10	中等度	使用中	77	2	3	3	2	2	2	1	1	1	2	改善	無	安全	有効	○	
20	女性	37	PLE				中等度	使用中	14	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(2)	(やや改善)	無	(—)	(—)	×	中止(使用感不良)
21	女性	35	PLE	B			軽度	使用中	77	1	1	3	1	3	1	1	1	1	1	改善	無	安全	有効	○	
22	女性	37	PLE				軽度	使用中	73	1	1	2	1	2	1	1	1	1	1	改善	無	安全	やや有効	○	
23	男性	69	CAD	AB			中等度	使用中	70	2	3	4	1	3	1	2	2	1	2	改善	無	安全	やや有効	○	
24	女性	74	PLE	—			軽度	未使用	63	3	2	4	1	2	1	1	1	1	1	著しく改善	無	安全	有効	○	
25	男性	80	CAD	AB	2.7	0.1668	高度	未使用	85	2	3	3	2	1	1	1	2	2	1	著しく改善	無	安全	有効	○	
26	男性	63	日光蕁麻疹	A			中等度	未使用	68	1	1	4	1	1	1	1	2	1	1	改善	無	安全	やや有効	○	
27	男性	63	CAD	AB			中等度	使用中	77	3	2	4	1	2	2	1	3	1	1	改善	無	安全	やや有効	○	

28	男性	80	CAD, 外因性光線過敏症	AB			中等度	使用中	85	3	3	4	2	1	2	2	2	1	1	改善	無	安全	有効	○	
29	女性	61	CAD				中等度	使用中	85	4	4	4	3	2	3	2	1	1	1	改善	無	安全	やや有効	○	
30	男性	75	CAD				中等度	未使用	70	1	2	5	5	3	1	1	2	2	2	改善	無	安全	やや有効	○	
31	男性	61	CAD	A		2	中等度	使用中	42	2	2	2	2	1	3	3	4	4	1	悪化	無	安全	無効	×	遮光有効性無効
32	男性	69	CAD	AB	10	3	中等度	使用中	70	4	4	4	2	4	2	1	2	1	2	著しく改善	無	安全	有効	○	
33	男性	68	CAD	AB	30	6	高度	使用中	70	1	1	1	1	1	1	1	1	1	1	不変	無	安全	有効	○	
34	男性	68	CAD	不明	不明	不明	軽度	未使用	70	1	1	3	3	3	1	1	1	1	1	著しく改善	無	安全	有効	○	
35	男性	73	CAD	A	60	6	中等度	未使用	71	(3)	(3)	(5)	(2)	(4)	(3)	(3)	(5)	(2)	(4)	(不変)	(無)	(安全)	(—)	(—)	逸脱
36	女性	46	PLE				中等度	使用中	77	2	2	3	1	1	2	1	2	1	1	改善	無	安全	やや有効	○	
37	女性	40	外因性光線過敏症				軽度	未使用	73	2	1	3	2	1	1	1	2	1	1	やや改善	無	安全	やや有効	○	
38	女性	35	PLE				中等度	未使用	77	3	3	3	2	3	2	2	3	2	2	やや改善	無	安全	やや有効	○	
39	女性	40	全身性エリテマトーデス	B	不明	不明	軽度	使用中	70	2	1	3	3	1	2	1	2	2	1	改善	無	安全	有効	○	

（　）データは不採用データ

した。また，2回目以降の観察時に，有害事象の発現の有無，試験終了時に，サンプルの回収の有無とアンケートの実施の有無を記入した。何らかの理由により試験期間内に試験を中止した場合は，中止日，中止理由，中止後の処置，経過を記載した。

5）有害事象

試験品塗布部の異常の有無を観察し，その結果を記録した。異常を認めた場合（有害事象）にはその発現日，症状および程度，試験品の継続使用の有無，判断の理由，有害事象の治療の有無等を記録した。症状の程度については，以下の5段階で評価した。A. 即座に中止し，症状が重い，B. 中止はしたが，症状は軽度または軽微，C. 中止はしたが，主観的症状のみ，D. 中止は無く，症状は軽度または軽微で継続使用が可能，E. 中止は無く，主観的症状のみであり，継続使用が可能

また，有害事象と試験品との因果関係についても考察を行い，以下の5段階での判断を実施した。0. 関連性なし，1. 関連性を否定できない，2. おそらく関連性あり，3. 関連あり，4. 不明

さらに，有害事象と試験品との関連性が疑われ，かつ，被験者の同意が得られた場合には，試験品との因果関係を明らかにするために，必要に応じて光パッチテストを実施することとした。

9．評価項目

試験終了時に，安全度と有効性についての総合評価を行った。

1）安全度

安全度の評価は副作用の出現，試験の継続等を基に下記の判定に従って4段階評価で行った。①安全である：副作用の出現がなかった，②ほぼ安全である：軽度の副作用が出現したが，無処置のまま継続使用が可能な程度だった，③やや問題あり：副作用が出現したが，処置を行い継続使用が可能であった，または，副作用は認められなかったが試験を中止した，④問題あり：副作用が出現し，使用を中止した

2）有効性

試験品の紫外線防御に関する有効性の評価は，試験開始前の日焼け止め料の使用の有無に分けて，**Table 3** の判定に従って4段階評価で行った。

10．アンケート調査

試験終了時に被験者に対して皮膚状態，試験品の使用感，試験開始前の日焼け止め料の使用の有無と使用製品名等についてアンケート調査を実施した。

11．結果の集計と統計解析

1）主要評価項目

安全度について，「①安全である」と「②ほぼ安全である」と評価された被験者の割合を試験品の安全度として算出した。ただし，使用感の悪さなどを理由に試験を中止した症例は試験品の安全性を評価するのに十分な期間使用出来ていないという理由から安全度の算出対象から除外した。また，逸脱症例についても，正しい使用法を行っていなかったという理由から同じく安全度の算出対象から除外した。