

ORIGINAL ARTICLE

# Prevention of photosensitivity with action spectrum adjusted protection for erythropoietic protoporphyria

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## ABSTRACT

Erythropoietic protoporphyria is a genetic disease characterized by sensitivity to sunlight caused by the accumulation of protoporphyrin IX. Photoprotection against ultraviolet A and visible light is necessary for erythropoietic porphyria patients because the absorption spectrum of protoporphyrin IX lies in both ultraviolet A and visible light region. We developed a novel index, *in vitro* porphyrin protection factor, based on the protoporphyrin IX absorbance spectrum. We also selected appropriate photoprotective products designed according to protoporphyrin IX absorbance. The porphyrin protection factors of a combination of make-up base with a powder as well as with a liquid foundation were significantly higher than those of a conventional sunscreen product, even at a small application dose. An in-use test carried out for 6 months showed that the efficacy of these products was 78.3%, and no adverse reactions were observed. Male subjects preferred liquid foundation, whereas all female subjects used powder foundation. The preference of the subjects could lead to the long-term use of the tested products. In conclusion, this study provided a new approach to improve photoprotection in erythropoietic protoporphyria patients.

**Key words:** cosmeceuticals, erythropoietic protoporphyria, photosensitivity disorder, protoporphyrin, sunlight.

## INTRODUCTION

Porphyrias are metabolic disorders caused by the deficiency of a specific enzyme in the heme biosynthetic pathway. They are classified as acute and cutaneous porphyrias based on the clinical manifestations.<sup>1–6</sup> Erythropoietic protoporphyria (EPP; Online Mendelian Inheritance in Man no. 177000) is an autosomal dominant disease caused by the decreased activity of ferrochelatase (FECH; E.C. 4.99.1.1), which is the terminal enzyme in the heme biosynthetic pathway.<sup>1–6</sup> A decrease in FECH activity results in the overproduction of protoporphyrin IX, leading to skin photosensitivity and liver dysfunction.<sup>7–9</sup>

Photoprotection is the most essential and effective approach to avoid protoporphyrin IX activation and the resultant painful symptoms, such as skin burn and erythema. EPP patients must either wear a hat and clothing that covers their entire body when going out in the sun or remain indoors during the daylight hours.<sup>4,10</sup> These restrictions limit their social activities and decrease their quality of life.

Sunscreens are effective in shielding the skin from ultraviolet (UV) light. However, the application of sunscreens does not completely prevent protoporphyrin IX activation because their protection efficacies for the visible light region are limited.

Photoprotective cosmetics that cover the UV-A and visible light regions are necessary for EPP patients. Therefore, we developed a new index of photoprotection calculated based on the absorbance of protoporphyrin IX measured *in vitro*. An in-use test was also carried out in EPP patients to assess the long-term efficacy and safety of the appropriate products selected based on the new index.

## METHODS

### Transmittance measurement

The transmittance of the samples placed on a poly methyl methacrylate plate (Schönberg, Hamburg, Germany) was measured in the UV-visible wavelength region (300–450 nm) by using an integrating sphere spectrophotometer (JV-2000; Labsphere, North Sutton, NH, USA).

### Calculation of *in vitro* porphyrin protection factor

The *in vitro* porphyrin protection factor (PPF) was calculated to determine the protection efficacy of a photoprotective product based on protoporphyrin IX absorbance. Briefly, the absorbance of the samples was multiplied by the absorbance of protoporphyrin IX at each wavelength and integrated to yield

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the area. To measure protoporphyrin IX absorbance, protoporphyrin IX ( $\geq 95\%$  purity; ALX-430-041; Enzo Life Science, Farmingdale, NY, USA) was dissolved in dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO, USA) to a final concentration of 2.5  $\mu\text{g}/\text{mL}$ . The light absorption between 300–450 nm was measured using a spectrophotometer (U-3010; Hitachi, Tokyo, Japan).

Porphyrin protection factor was designated as the ratio of the two integrals as shown in the following equation:

$$\text{PPF}_{in\ vitro} = \frac{\int_{\lambda=300\text{ nm}}^{\lambda=450\text{ nm}} PP(\lambda) I(\lambda) d(\lambda)}{\int_{\lambda=300\text{ nm}}^{\lambda=450\text{ nm}} PP(\lambda) I(\lambda) 10^{-A(\lambda)} d(\lambda)}$$

where  $PP(\lambda)$  is the mean absorbance of protoporphyrin IX solution,  $I(\lambda)$  is the standard spectral irradiance of the solar source based on an air mass of 1.5 G,<sup>11</sup> and  $A(\lambda)$  is the mean absorbance calculated from the transmittance data for the test product.

### Products tested

The commercially available samples chosen according to protoporphyrin IX absorbance for this study are as follows: one make-up base emulsion, three powder foundations (A, B and C), one liquid foundation, and a sunscreen product, manufactured by KOSÉ (Tokyo, Japan). The sun protection factor (SPF) and protection factor of UV-A (PFA) of the samples were assessed according to the international SPF test methods<sup>12</sup> and the method of the Japan Cosmetic Industry Association,<sup>13</sup> respectively. The test samples contained different absorbents. The make-up base emulsion (SPF 26.5 and PFA 5.0) was an oil-in-water emulsion containing ethylhexyl methoxycinnamate, titanium dioxide and iron oxide. Powder foundation A (SPF 15.0 and PFA 6.7) contained ethylhexyl methoxycinnamate, benzophenone-3, titanium dioxide and iron oxide, whereas samples B (SPF 28.1 and PFA 7.7) and C (SPF 21.1 and PFA 4.4) contained ethylhexyl methoxycinnamate, titanium dioxide and iron oxide. The liquid foundation (SPF 28.1 and PFA 4.9) was an oil-in-water emulsion containing ethylhexyl methoxycinnamate, titanium dioxide and iron oxide. The sunscreen (SPF 64.4 and PFA 9.1) was a water-in-oil emulsion containing zinc oxide, ethylhexyl methoxycinnamate and phenylbenzimidazole sulfonic acid.

### In-use test in Japanese EPP patients

The in-use test was conducted under the supervision of physicians at three clinics (Kindai University Faculty of Medicine, The Jikei University School of Medicine and Kanazawa Red Cross Hospital) in accordance with the principles of the Declaration of Helsinki. Prior to the study, written informed consent was obtained from each participant. Twenty-three Japanese EPP patients participated in the in-use test. The subjects were diagnosed with EPP on the basis of photosensitivity and abnormally elevated levels of protoporphyrin in erythrocytes; in most cases, the diagnosis was confirmed by gene analysis. The patients selected the appropriate products by themselves,

which were either a combination of make-up base emulsion and powder foundation (sample A) or make-up base emulsion and liquid foundation. During the in-use test, the patients were instructed by the physicians to always apply the products consistently, with a thickness of at least 0.5  $\text{mg}/\text{cm}^2$ , which represented one fingertip unit, for the make-up base emulsion or liquid foundation. For the powder foundation, the patients were instructed to apply the product at least five times to the face using a powder puff, at a thickness of at least 0.2  $\text{mg}/\text{cm}^2$ . The patients applied the product thoroughly, not leaving any uncoated area on their face and body, before going outside. They applied the product once or twice a day depending on their exposure time. The patients were inquired regarding their consistency of application and the time after which they started to experience painful itching, tingling or burning sensation during the in-use test. In pediatric subjects, we asked their parents to visit the clinics and instructed them how to use the products on their children. The consistency of application and the time after which the pediatric patients started to experience the symptoms were confirmed by their parents.

Patients who followed the aforementioned instructions for at least 6 months were included in the study.

The overall assessment of effectiveness was classified into four grades: “effective”, no appearance of erythema and edema; “somewhat effective”, decrease in frequency of erythema and edema; “unchanged”, no change in frequency of erythema and edema; and “worsened”, increase or aggravation of erythema and edema as judged by the physicians’ comprehensive evaluation based on clinical and subjective symptoms. The improvement was assessed through comparison of the effectiveness before and after the application of the product. Most EPP patients in our study claimed photosensitivity from April to September, but not from October to March. In this study, we evaluated the efficacy of the product from April to September.

### Data analysis

The mean PPF for the tested cosmetic products were compared with that of the sunscreens by using Dunnett’s test. The time after which the patients started to experience photosensitivity was compared using a paired *t*-test. *P*-values of less than 0.05 were considered to be statistically significant.

## RESULTS

### PPF for powder foundations

The PPF were calculated based on the mean absorbance spectrum of protoporphyrin IX from 300–450 nm (Fig. 1).

The three types of powder foundations (A, B and C) with different ingredients or absorbents were compared with each other. All the three samples (A, B and C) had different transmittance spectra in the UV and visible light regions, which indicated different protection efficacies in these regions (Fig. 2). The SPF, PFA and PPF of the three powder foundations are summarized in Table 1. The PPF for samples A, B and C at a thickness of 0.2  $\text{mg}/\text{cm}^2$  were 1.8, 1.6 and 1.4, respectively. Although sample B had the highest SPF (28.1) and PFA (7.7)

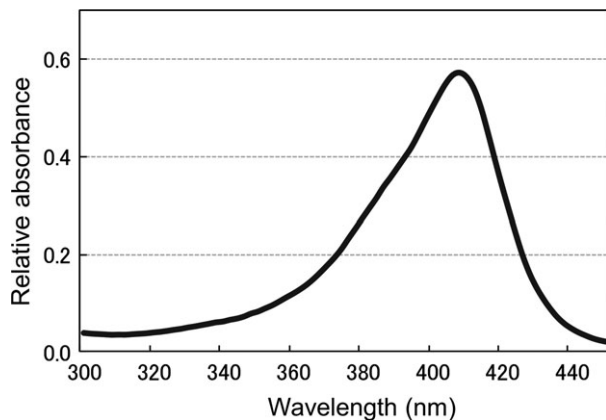


Figure 1. Absorbance spectrum of protoporphyrin IX.

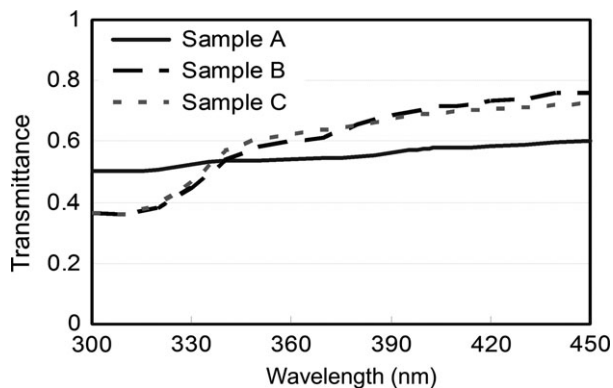


Figure 2. Transmittance spectra of three powder foundations.

Table 1. Porphyrin protection factors (PPF), sun protection factors (SPF) and protection factors of ultraviolet A (PFA) for three powder foundations

	Powder foundation		
	Sample A	Sample B	Sample C
PPF	1.8	1.6	1.4
SPF	15.0	28.1	21.1
PFA	6.7	7.7	4.4

values, sample A had the highest PPF. This indicated that PPF is independent of SPF and PFA. Transmittance in the visible light region, especially at 408 nm, influenced the PPF. The sample with the highest PPF (sample A) was selected for the in-use test in EPP patients. Liquid foundation samples having similar transmittance spectra were selected and used for in-use testing.

#### PPF for combination of cosmetics

The PPF for the combinations of cosmetic products were compared with those of sunscreen to determine whether the combinations provided sufficient protection efficacy even if the

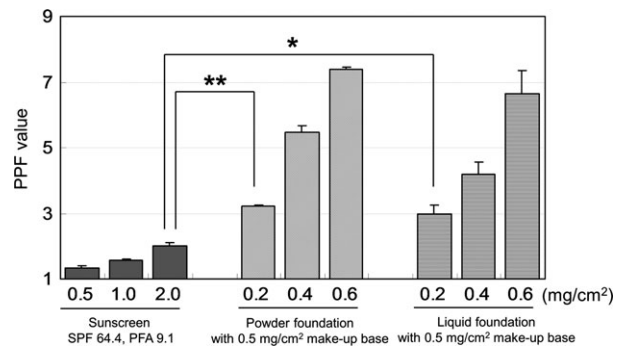


Figure 3. Relationship between the application amount of the product and porphyrin protection factor (PPF). Sunscreen was applied at a thickness of 0.5, 1.0 or 2.0 mg/cm<sup>2</sup>, and powder (sample A) or liquid foundation was applied at 0.2, 0.4 and 0.6 mg/cm<sup>2</sup>, with make-up base emulsion at a thickness of 0.5 mg/cm<sup>2</sup>. Error bars represent the standard error. Dunnett's test, \* $P < 0.01$ ; \*\* $P < 0.001$ .

application amount was small (Fig. 3). The application of the powder foundation sample A at a thickness of 0.2, 0.4 and 0.6 mg/cm<sup>2</sup> and make-up base emulsion at a thickness of 0.5 mg/cm<sup>2</sup> resulted in PPF of  $3.22 \pm 0.05$ ,  $5.46 \pm 0.21$  and  $7.37 \pm 0.09$ , respectively. The application of liquid foundation at 0.2, 0.4 and 0.6 mg/cm<sup>2</sup> thickness and make-up base emulsion at a thickness of 0.5 mg/cm<sup>2</sup> resulted in PPF of  $2.97 \pm 0.27$ ,  $4.21 \pm 0.36$  and  $6.64 \pm 0.72$ , respectively. The application of powder or liquid foundation onto the make-up base emulsion increased the PPF in a dose-dependent manner. The application of sunscreen at a thickness of 0.5, 1.0 and 2.0 mg/cm<sup>2</sup> resulted in PPF of  $1.35 \pm 0.07$ ,  $1.58 \pm 0.01$  and  $2.01 \pm 0.09$ , respectively. The powder ( $P < 0.001$ ) or liquid ( $P < 0.01$ ) foundations combined with the make-up base emulsion at an application thickness of 0.2 mg/cm<sup>2</sup> for each foundation and 0.5 mg/cm<sup>2</sup> for the make-up base emulsion showed significantly higher PPF than those of the sunscreen at an application thickness of 2.0 mg/cm<sup>2</sup> (Fig. 3).

#### Clinical evaluation in EPP patients

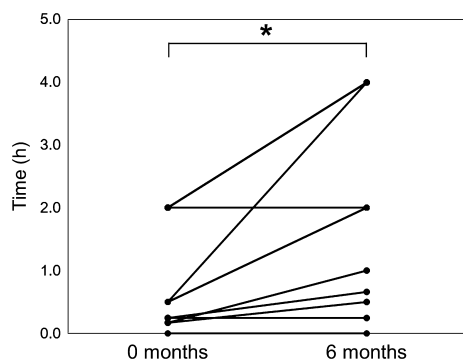
An in-use test was conducted in 23 Japanese EPP patients aged 7–66 years (mean, 24). The erythrocytic protoporphyrin level of EPP patients was measured before the in-use test (Table 2). All the patients chose a combination of make-up base emulsion and either powder or liquid foundation or both. They used the products for at least 6 months. Many male subjects (66.7%) favored the combination of make-up base emulsion and liquid foundation, whereas all female subjects chose the combination of make-up base emulsion and powder foundation. Seven male patients (46.7%) changed the samples from powder to liquid foundation during the testing period according to their requirement (Table 2).

The time of sun exposure when patients began to experience symptoms, such as pain, itching or redness, was prolonged significantly by the application of products in nine subjects, approximately 2–8 times, compared with that without application (Fig. 4). We performed statistical analysis and found

**Table 2.** Study population characteristics in the in-use test

No.	Sex	Age (years)	Product used	Concentration of erythrocytic PP ( $\mu\text{g}/\text{dL}$ )
1	Male	9	MBE, PF	721
2	Male	13	MBE, PF, LF	1895
3	Male	14	MBE, LF	1180
4	Male	15	MBE, PF, LF	1157
5	Male	15	MBE, PF, LF	1068
6	Male	16	MBE, PF, LF	1410
7	Male	16	MBE, PF	3857
8	Male	17	MBE, PF, LF	1531
9	Male	19	MBE, PF, LF	2220
10	Male	19	MBE, PF	1871
11	Male	19	MBE, LF	3618
12	Male	20	MBE, LF	2141
13	Male	21	MBE, PF	2013
14	Male	39	MBE, PF	217
15	Male	58	MBE, PF, LF	2034
16	Female	7	MBE, PF	4487
17	Female	12	MBE, PF	1421
18	Female	12	MBE, PF	1208
19	Female	16	MBE, PF	67
20	Female	26	MBE, PF	496
21	Female	29	MBE, PF	1785
22	Female	39	MBE, PF	1130
23	Female	40	MBE, PF	1229

Concentration of blood protoporphyrin IX was measured before the study. LF, liquid foundation; MBE, make-up base emulsion; PF, powder foundation; PP, protoporphyrin.



**Figure 4.** Duration of sun exposure when the patients began to experience photosensitivity symptoms. Paired *t*-test,  $*P < 0.01$ .

no statistically significant differences between the effect (prolongation of time until the induction of symptoms) and age, concentration of erythrocytic protoporphyrin or the severity of the skin in this study. However, a positive trend was observed between the effect and the severity of the skin.

The efficacy of the powder or liquid foundation applied onto the make-up base emulsion is shown in Table 3. In response to this evaluation, 43.5% of the subjects were classified as effective, 34.8% as somewhat effective and 21.7% as

**Table 3.** Overall assessment of effectiveness by physicians

Rating	No. of patients	%
Effective	10	43.5
Somewhat effective	8	34.8
Unchanged	5	21.7
Worsened	0	0

unchanged. Worsening of symptoms and adverse reactions were not observed in any subjects.

## DISCUSSION

A previous study reported that the topical application of opaque and physical sunscreen is effective in preventing photosensitive reactions in EPP patients by protecting not only against UV-A, but also against the visible light region.<sup>14</sup> However, such high-shielding products render an unnatural color tone or white residue on the skin.

Moseley *et al.*<sup>15</sup> developed a protection index of sunscreen products for photosensitive patients with porphyria cutanea tarda. In their method, the patient's skin was irradiated with light to determine their index based on erythema reaction. The novel index PPF developed in our study is calculated from the absorbance of protoporphyrin IX as the action spectrum for EPP patients in accordance with the SPF measurements proposed by Sayre *et al.*<sup>16</sup>

Our study revealed that PPF was different for each powder foundation sample. Moreover, SPF and PFA were not correlated with PPF. Although SPF or PFA of the foundation products was much lower than that of the sunscreen, the in-use test indicated that foundation products with higher PPF were effective in EPP patients. Therefore, SPF and PFA are considered inadequate, whereas PPF is regarded as an ideal protection index for EPP patients. However, PFA may have some preventive effects on sensitivity because UV-A contributed, to a certain extent, to protection against EPP, particularly in the wavelength range 370–400 nm, if 2 mg/cm<sup>2</sup> of product is applied.

We demonstrated that the PPF values of make-up base emulsion and foundations increased in a dose-dependent manner. In addition, the PPF values of these combinations, even for a small quantity, were higher than those of sunscreen. Therefore, foundation products are more effective than sunscreen products in protecting the visible light region even in real use conditions.<sup>17–19</sup>

Moreover, we performed an in-use study in 23 Japanese EPP patients to confirm the efficacy and safety of the combination of make-up base emulsion and powder or liquid foundation selected after PPF measurement. The products tested were not effective in 21.7% of patients, whereas these products showed effectiveness in 78.3% of patients. The products with PPF 1.4–1.8 in this study might have not been effective in more sensitive patients that could be non-effective in 21.7% of patients. On the other hand, these products might have been effective in less sensitive patients that could be effective in 78.3% of patients. The product with higher PPF may be effective in non-effective patients.

No adverse events were observed for the products. The patients chose the type and color tone of the foundations according to their preference. Different trends were observed in the product choice between male and female subjects. Many female subjects chose powder foundation. This might have been because they focused on the well-finished texture that could make them look more attractive. However, all male subjects used liquid foundations as they might have focused on a natural look, which let them continue the use of the product for a long period without any complaints. They had not used make-up products before this study. Therefore, the most important factors influencing the long-term use of the products are thought to be a well-considered formulation providing a natural look as well as concordance with the patient's preferences. The duration time required to experience photosensitivity increased with an increase in the severity of the skin with no significant differences. We would like to clarify this trend with increasing patients in a future study.

In conclusion, we provided a new approach to reduce photosensitivity in EPP patients. PPF may be an important indicator for giving proper guidance to EPP patients. However, the products should be carefully selected based on the PPF required, under the guidance of physicians. We will continue to develop more effective and suitable foundation products based on PPF and contribute to the improvement of quality of life in EPP patients.

**CONFLICT OF INTEREST:** Takashi Teramura, Makoto Mizuno, Hajime Asano and Eiji Naru are employees of KOSÉ Corporation, which markets cosmetics containing foundation and sunscreen products. Shigeru Kawara, Ryoichi Kamide and Akira Kawada have no conflict of interests to declare.

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