







ORIGINAL ARTICLE

Bioactive substances in the stratum corneum of the epidermis found as indicators of skin damage due to sun exposure

Atsushi Fukunaga¹  | Satoshi Fukushima²  | Hiroaki Iwata³ | Makiko Nakahara⁴  | Rikako Sasaki⁵ | Naoko Baba⁶ | Hiroshi Matsunaka⁷  | Yumi Murakami⁷  | Masutaka Furue⁴  | Chikako Nishigori¹

¹Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, Kobe, Japan

²Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

³Department of Dermatology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

⁴Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

⁵Nashinohana Dermatological Clinic, Tokyo, Japan

⁶Department of Dermatology, Kanagawa Children's Medical Center, Kanagawa, Japan

⁷NOV Academic Research, TOKIWA Pharmaceutical Co., Ltd, Tokyo, Japan

Correspondence

Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, Kobe 650-0017, Japan.

Email: atsushi@med.kobe-u.ac.jp

Funding information

Tokiwa Pharmaceutical Co., Ltd.

Abstract

Background/Purpose: Although an inflammatory response upon acute injury caused by ultraviolet radiation (UV) can be observed immediately, the influence of long-term, repetitive low-dose UV exposure on the skin cannot be precisely perceived, making early detection of chronic damage difficult. This study investigated bioactive substances in the stratum corneum as a potential early and sensitive indicator of the influence of sun exposure on the skin using receiver operating characteristic (ROC) analysis.

Methods: Receiver operating characteristic analysis was performed to assess the responsiveness of cytokines [interleukin (IL)-1 α , IL-1 receptor antagonist (IL-1ra), IL-10, tumor necrosis factor (TNF)- α], BCL2-associated protein X (Bax), Toll-like receptor (TLR)3, and TLR4 in the stratum corneum of healthy people exposed (dorsum of the hand) and unexposed (inner arm) to UV. Sunscreen was applied to patients with photodermatosis for 4 weeks to evaluate changes in IL-1ra/IL-1 α , TNF- α , Bax, and TLR3 levels after sunscreen application, as these molecules exhibited high responsiveness to sun exposure according to ROC analysis. In addition, IL-1ra, IL-1 α , and IL-10 levels were quantified by enzyme-linked immunosorbent assay, and TNF- α , Bax, TLR3, and TLR4 levels were semi-quantitatively assessed by immunocytochemistry.

Results: Receiver operating characteristic analysis identified IL-1ra/IL-1 α , TNF- α , Bax, and TLR3 in the stratum corneum as highly responsive to sun exposure. Moreover, in participants, including patients with photodermatosis, IL-1ra/IL-1 α , TNF- α , and Bax levels decreased significantly after sunscreen application.

Conclusion: The results revealed that IL-1ra/IL-1 α , TNF- α , and Bax in the stratum corneum represent sensitive indicators of the influence of sun exposure on the skin.

KEYWORDS

Bax, IL-1ra, IL-1 α , ROC analysis, TLR3, TNF- α

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. Photodermatology, Photoimmunology & Photomedicine published by John Wiley & Sons Ltd.

1 | INTRODUCTION

The skin is an organ that serves as the boundary between the body and the external environment and functions as a barrier that prevents the entry of various stimuli and foreign substances. The barrier function of the skin can be affected by exogenous stimuli, among which ultraviolet radiation (UV) from the sun is the most significant. UV exposure stimulates the release of various cytokines from keratinocytes at the epidermal level, resulting in inflammation and pigmentation. At the dermal level, the skin undergoes short- and long-term effects of UV in the form of degeneration of collagen fibers by fibroblastic production of degrading enzymes.¹⁻³ Short-term excessive exposure to UV results in acute inflammation (commonly known as sunburn) and subsequent suntan, which occurs as pigmentation. Low-dose UV exposure that does not cause an inflammatory response can still cause aging (photoaging) effects, resulting in spots and wrinkles if the exposure is chronic and repetitive, as well as skin cancer.^{4,5} Acute damage caused by UV is easily recognized as sunburn and suntan, but it is more difficult to recognize chronic damage related to UV exposure at an early stage; thus, a prompt and sensitive indicator of the influence of UV on the skin is required.

Human skin presents increases in tumor necrosis factor (TNF)- α levels after several hours of UV exposure and increased interleukin (IL)-1 α and IL-10 levels after >10 hours of UV exposure.⁶ Moreover, the parts of the body chronically exposed to UV present significantly higher IL-1 receptor antagonist (IL-1ra) and IL-1ra/IL-1 α levels relative to unexposed parts.⁷ In addition, Toll-like receptor (TLR)3 reportedly exacerbates UV-induced skin damage and radiation enteritis.⁸ Furthermore, UV exposure induces the production of inflammatory cytokines, resulting in acute and chronic inflammation, and a previous study suggests that TLR4 plays an important role in linking inflammation to cancer.⁹ Bcl-2-associated X protein (Bax) is also reported to be involved in p53-mediated apoptosis in UV-induced *P53* gene mutations.¹⁰

Patients with photodermatosis and photoexacerbated conditions, such as systemic lupus erythematosus (SLE), must be protected from sunlight daily.^{11,12} Healthy individuals should also protect themselves from sunlight in order to prevent both acute and chronic damage starting in childhood and throughout their life.^{13,14} Methods of skin protection from UV exposure include wearing long-sleeved clothing, hats, gloves, and parasols, as well as sunscreen application. The continuous use of these appropriate methods of sun protection should improve and prevent exacerbation of symptoms of photodermatosis and photoaging, with the efficacy of these methods commonly acknowledged.¹⁵⁻¹⁸

This study investigated the potential early indicators of the effects of sun exposure on the skin. We assessed common bioactive substances in the stratum corneum, which are under- or overexpressed in response to sun exposure in parts of the body exposed and unexposed to sunlight, using receiver operating characteristic (ROC) analysis. Additionally, we determined whether these bioactive substances that responded to UV exposure would serve as indicators by observing the effect of sunscreen application on patients with photodermatosis and photoexacerbated diseases.

2 | MATERIALS AND METHODS

The following tests were performed targeting IL-1ra, IL-1 α , IL-10, Bax, TNF- α , TLR3, and TLR4 as parameters that reportedly fluctuate in epidermal keratinocytes in response to UV exposure and can be detected in the stratum corneum.

2.1 | Measuring bioactive substances in the stratum corneum of healthy individuals upon sun exposure

2.1.1 | Participants

Participants included healthy individuals aged ≥ 20 years, without any underlying diseases, and who wore long-sleeved shirts throughout the year. The dorsum of the hand and inner arm were selected as exposed and unexposed body parts, respectively. This study was approved by the Yoyogi Mental Clinic Ethics Review Board (N049). All participants were informed of the aims and methods of the study and provided their written consent.

2.1.2 | Collection of stratum corneum samples by tape stripping

After wiping the dorsum of the hand and inner arm, stratum corneum was collected once by attaching and peeling a strip of 24 mm \times 100 mm adhesive tape (CELLOTAPE™; Nichiban).

2.1.3 | Enzyme-linked immunosorbent assay (ELISA)

The 24 mm \times 60 mm strips of tape with adhered stratum corneum samples were cut into ~ 5 -mm² pieces and immersed in 1 mL phosphate-buffered saline (PBS) and sonicated for 30 seconds on ice to obtain the extract. Samples were then centrifuged at 4°C and 16 100 g for 10 minutes to eliminate the insoluble fraction, and the supernatant was used for further analysis as the stratum corneum extract. Total protein content in supernatant was measured using a Quick Start protein assay (Bio-Rad Laboratories), with bovine serum albumin (BSA) used as a reference. The levels of IL-1 α , IL-1ra, and IL-10 in the supernatant were measured using respective ELISA kits (R&D Systems). The amount of target proteins per total protein content was calculated, and the IL-1ra/IL-1 α ratio was calculated, as previously described.¹⁹

2.1.4 | Immunocytochemistry

Biologically active substances that could not be quantified by ELISA due to their low amounts in the stratum corneum were measured by immunocytochemistry. According to a previously described method,²⁰ sample tape strips were pasted onto a glass slide to transfer the

corneal cells by immersing them in xylene overnight. The cells were subsequently fixed with cold acetone, washed with PBS containing 0.3% Triton X-100 (PBST), and blocked with 0.3% Triton X-100 and 1% BSA containing 0.1% NaN_3 (1% BSA in PBST; Vector Laboratories). The primary antibody was added overnight at 4°C, and samples were washed with PBST and then reacted at room temperature for 2 hours with fluorescence-labeled secondary antibodies (Invitrogen). Primary antibodies included anti-TNF- α (Sigma-Aldrich), anti-Bax (Proteintech), anti-TLR3 (Abcam), and anti-TLR4 (Santa Cruz Biotechnology). To detect Bax levels, samples were treated with 10 mmol/L citrate buffer (pH 6.0) at 98°C for 20 minutes and then reacted with the antibody. In the control experiments, the primary antibodies were replaced by normal IgG (Abcam). After the secondary reaction, cells were washed with PBS and mounted on a cover glass for observation under a fluorescence microscope (Keyence). Image analysis was performed using Photoshop software (Adobe Systems Incorporated) to calculate the fluorescence intensity per cellular area.

2.1.5 | Statistical analysis

The Mann-Whitney *U* test and ROC analysis were performed to determine the significance of differences in the measured levels of IL-1ra/IL-1 α , Bax, TNF- α , TLR3, TLR4, and IL-10 between exposed and unexposed stratum corneum. SPSS software (v. 27.0; IBM Corp.) was used for the analyses, and a *P* < .05 was considered statistically significant.

2.2 | Measurement of the indicators after sunscreen application

2.2.1 | Participants

Participants were patients with photodermatosis [eg, photosensitive dermatitis (of unknown cause), chronic actinic dermatitis, xeroderma pigmentosum, and drug-induced photodermatosis], photoexacerbated diseases (eg, SLE and dermatomyositis), dermatitis with dryness (atopic dermatitis and other diseases in which avoiding sun exposure is considered desirable for treatment), and other diseases (eg, solar keratosis). However, patients meeting the following criteria were excluded: previous experience of severe skin symptoms associated with sunscreen use; presence of a lesion at the evaluation site that was unsuitable for sunscreen application; complications or undergoing treatment that might affect sunscreen application; unable to be examined 4 weeks later; or otherwise deemed ineligible to participate in this trial by the attending physician.

2.2.2 | Institutions

This study was conducted in six facilities in Japan (Kobe University Hospital, Hokkaido University Hospital, Kyushu University

Hospital, Kumamoto University Hospital, Kanagawa Children's Medical Center, and Nashinohana Dermatological Clinic). Before starting this trial, the protocol was approved by the institutional review boards of the participating facilities (Kobe University Hospital: 1575, Hokkaido University Hospital: 013-0286, Kyushu University Hospital: 25086, Kumamoto University Hospital: advance 1811, and Kanagawa Children's Medical Center: 83-01). The trial at Nashinohana Dermatological Clinic was approved by the Hattori Clinic Ethics Review Board. All trial participants were informed of the aims and methods of the study and provided their written consent.

2.2.3 | Sunscreens

The sunscreens used in this study did not contain UV absorbers, contained titanium oxide and zinc oxide as UV reflectors (Noevir Co., Ltd.), and were as follows: lotion type, "NOV[®] UV LOTION EX" SPF32, PA+++; W/O emulsified cream type, "NOV[®] UV SHIELD EX" SPF50+, PA++++; O/W emulsified milk type, "NOV[®] UV MILK EX" SPF32, PA+++; stick-type (skin color and foundation type), "NOV[®] UV STICK EX" SPF50+, and PA++++ with high visible light blocking effect.

2.2.4 | Application method

Sunscreen was applied to the body parts exposed to the sun, such as the face, for 4 weeks. The attending physician selected one or more sunscreens from the four described products depending on the skin condition, severity of sun exposure, and preference of the participant. A stick-type sunscreen was used in combination for participants that required blocking of visible light. Sunscreen (2 mg/cm²) was applied to the parts of the body exposed to sunlight in the morning regardless of whether the participant was going to go outdoors during the day. Participants were further instructed to reapply sunscreen every 3 hours on days when they were spending long hours outdoors. Internal and external drugs and products, including cosmetics, in use by participants remained unchanged during the study period. Sunscreens previously used by participants before the trial were switched to those selected by the attending physician.

2.2.5 | Observation and assessment

Observation and assessment of the stratum corneum were performed on the face (cheeks). The attending physician recorded participant identification code (linked and anonymized prior to the start of the trial), age, sex, diagnosis, disease duration, treatment history, complications, and drugs concomitantly used during the study period (drug name, dosage form, and dosage). Additionally, clinical skin findings of the face and stratum corneum sampled by

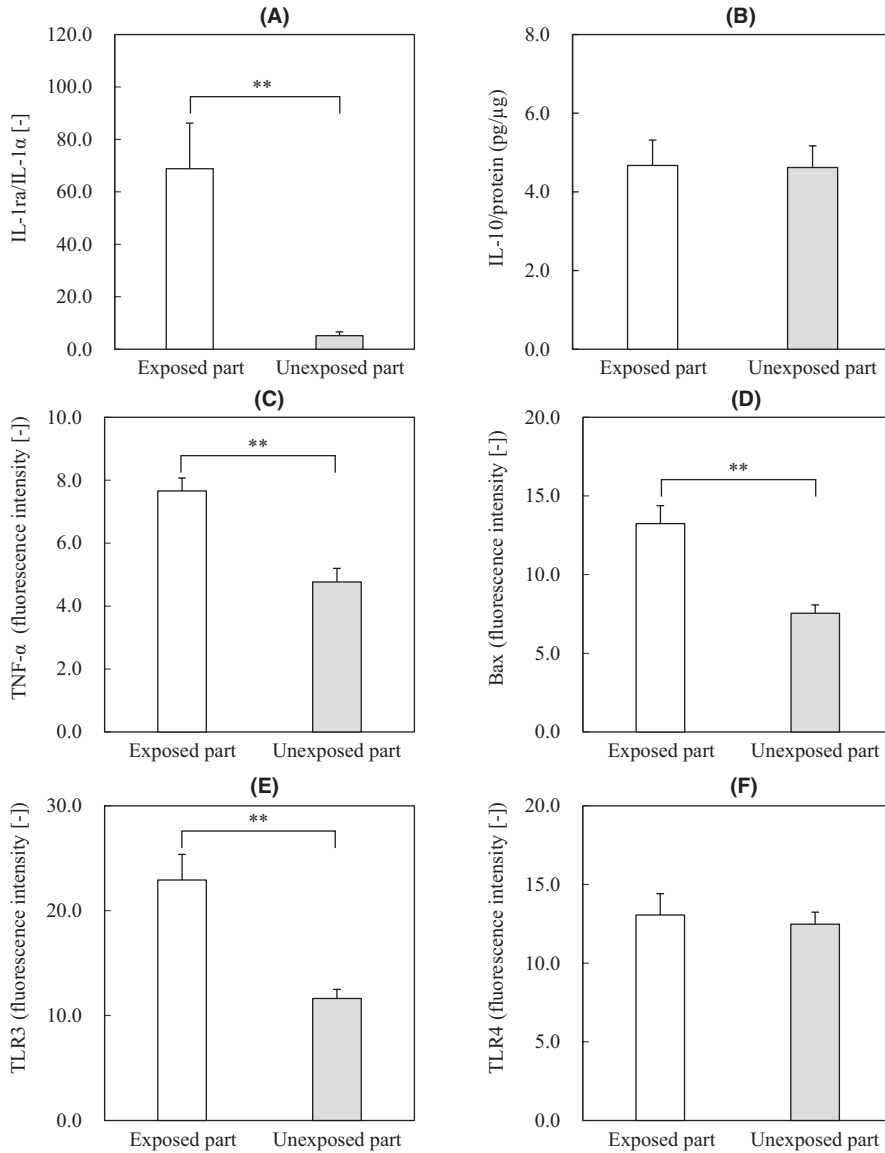


FIGURE 1 Comparison of bioactive substances in the stratum corneum of sun-exposed and -unexposed parts of the body in healthy individuals. Levels of (A) IL-1ra/IL-1α, (B) IL-10, (C) TNF-α, (D) Bax, (E) TLR3, and (F) TLR4. Exposed part: dorsum of the hand; and unexposed part: inner arm. Data represent that mean ± standard error of the mean. P-value was calculated using the Mann-Whitney U test (n = 14). **P < .01

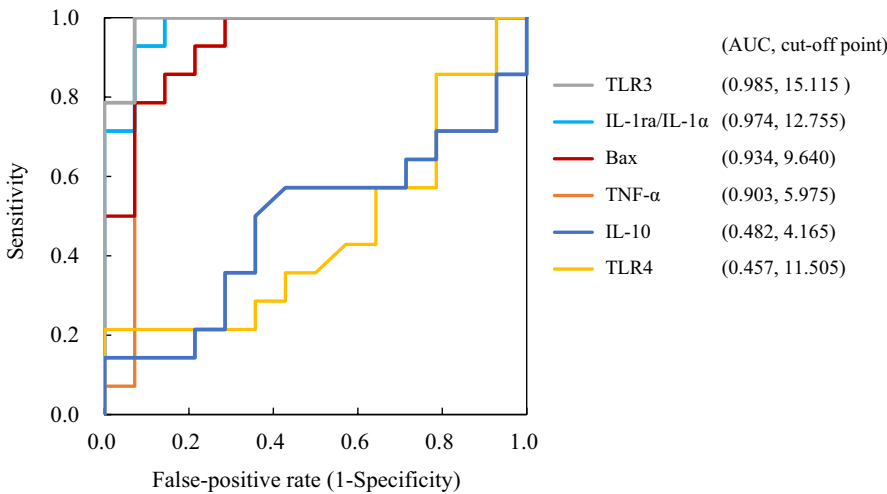


FIGURE 2 ROC curve of bioactive substances in the stratum corneum of sun-exposed and -unexposed parts of the body in healthy individuals. AUC, area under the curve

tape stripping were recorded once at the start and at the end of the study period. The degrees of erythema, itching, and dryness were assessed at the start and completion of the study period using

a 5-point scale (None: 0; Slight: 1; Mild: 2; Moderate: 3; Severe: 4). Items of analysis of the stratum corneum included those that showed a significant response to sun exposure according to ROC

TABLE 1 Participant background

	Photodermatosis	Photoexacerbated diseases	Dermatitis with dryness	Other diseases	All diseases		
Sex							
Male	21	6	27	3	57		
Female	15	31	29	4	79		
Total	36	37	56	7	136		
Age (y)							
0-9	2	1	29	1	33		
10-19	2	1	1	0	4		
20-29	1	4	4	1	10		
30-39	9	6	10	0	25		
40-49	4	10	7	2	23		
50-59	1	7	1	0	9		
60-69	9	6	2	1	18		
≥70	8	2	1	2	13		
Unknown			1		1		
Mean ± SD	49.6 ± 22.0	45.8 ± 15.9	20.4 ± 19.5	47.1 ± 26.1	36.5 ± 23.7		
Severity							
Slight	5	5	6	0	16		
Mild	9	10	26	4	49		
Moderate	17	17	22	3	59		
Severe	4	4	2	0	10		
Disease name							
Photosensitivity dermatitis (unknown cause)	20	Systemic lupus erythematosus	12	Atopic dermatitis	45	Solar keratosis	2
Chronic actinic dermatitis	6	Dermatomyositis	9	Pediatric dry eczema	11	Pollinosis dermatitis	1
Xeroderma pigmentosum	2	Acne rosacea	7			Pachyderma	1
Polymorphic light eruption	2	Sjogren's syndrome	4			Congenital bullous dermatosis	1
Solar eczema	2	Discoid lupus erythematosus	2			Cutaneous lymphoma	1
Drug-induced photodermatosis	2	Mixed connective-tissue disease	1			Oculocutaneous albinism	1
Protoporphyrria	1	Lupus erythematosus profundus	1				
Solar urticaria	1	Cutaneous lupus erythematosus	1				

analysis, as described in section 2.1 and performed according to sections 2.1.2 through 2.1.4.

2.2.6 | Statistical analysis

Scores at the start and completion of the study and level of bioactive substances in the stratum corneum were analyzed using the Wilcoxon signed-rank test. IBM® SPSS® Statistics Windows version 27.0 (IBM Corp.) was used for all statistical analyses, and a $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Response of bioactive substances in the stratum corneum to sun exposure in healthy individuals

The participants included 14 individuals (7 men and 7 women) with a mean age of 38.1 ± 10.7 years and no underlying diseases, such as atopic dermatitis. The dorsum of the hand and inner arm were assessed as sun-exposed and -unexposed parts, respectively. The levels

of IL-1ra/IL-1 α , TNF- α , Bax, and TLR3 in the exposed parts were significantly higher than those in the unexposed parts ($P < .001$, $P < .001$, $P < .001$, and $P < .001$, respectively) (Figure 1A, C-E). However, we observed no significant difference in IL-10 and TLR4 levels between exposed and unexposed parts (Figure 1B, F). The area under the ROC curve (AUC) for TLR3, IL-1ra/IL-1 α , Bax, TNF- α , IL-10, and TLR4 was 0.985, 0.974, 0.934, 0.903, 0.482, and 0.457, respectively (Figure 2).

3.2 | Validating indicators of sunscreen use in patients

The total of 136 participants (mean age: 36.5 ± 23.7 years) included 36 patients with photodermatitis (mean age: 49.6 ± 22.0 years), 37 with photoexacerbated diseases (mean age: 45.8 ± 15.9 years), 56 with dermatitis with dryness (mean age: 20.4 ± 19.5 years), and 7 with other diseases (mean age: 47.1 ± 26.1 years) (Table 1). After 4 weeks, scores for all observed parameters (erythema, itching, and dryness) significantly decreased in photodermatitis, photoexacerbated diseases, dermatitis with dryness, and all diseases as compared with those at the start ($P < .001$, $P < .001$, $P < .001$, and $P < .001$, respectively). No significant changes were observed for other diseases (Table 2).

Additionally, IL-1ra/IL-1 α , TNF- α , and Bax levels in photodermatitis decreased significantly, and that of TLR3 increased significantly after 4 weeks from study initiation ($P = .019$, $P = .038$, $P = .003$, and $P = .016$, respectively). For dermatitis with dryness, TNF- α and Bax levels decreased significantly, and TLR3 level increased significantly ($P = .006$, $P = .012$, and $P < .001$, respectively). In all diseases, IL-1ra/IL-1 α , TNF- α , and Bax levels decreased significantly, and the level of TLR3 increased significantly ($P = .016$, $P = .040$, $P = .005$, and $P < .001$, respectively) (Table 3).

4 | DISCUSSION

This study investigated whether common bioactive substances in the stratum corneum, the expression of which fluctuates in response to sun exposure, can function as indicators of skin damage upon sun exposure. IL-1ra/IL-1 α , TNF- α , Bax, and TLR3 levels in sun-exposed parts of the stratum corneum differed significantly from that in unexposed parts. In ROC analysis, an AUC from 0.9 to 1.0 is considered to indicate high accuracy⁷; thus, in the present study, the AUC > 0.9 determined for IL-1ra/IL-1 α , TNF- α , Bax, and TLR3 levels indicated their high response to sun exposure. Indeed, IL-1ra/IL-1 α , TNF- α , and Bax levels decreased significantly after sunscreen use in patients with photodermatitis, confirming that these bioactive substances in the stratum corneum could serve as indicators of the influence of sun exposure on the skin.

IL-1ra/IL-1 α and TNF- α levels reportedly increase in response to inflammation or UV exposure.^{21,22} Moreover, although cells that suffer UV-induced DNA damage can undergo apoptosis, there are no previous studies on Bax, a protein involved in inducing apoptosis,¹⁰ in the stratum corneum. The present study revealed a relationship between sun exposure and TLR3 and Bax levels in the stratum corneum in healthy individuals. However, further investigation is needed to determine whether these fluctuations cause skin damage or occur as a result of skin damage.

Since IL-1ra/IL-1 α , TNF- α , and Bax levels decreased significantly with the use of sunscreen in overall participants of this study including patients with photodermatitis, who required more rigorous protection from sunlight, it is possible that inflammation and apoptosis were inhibited by sun protection. Indeed, sun protection using sunscreens also improved clinical skin findings of the face in participants, including those with photodermatitis. TLR3 reportedly mediates the inflammatory response to UV⁸; therefore,

TABLE 2 Changes in clinical skin findings by the application of sunscreen

	Photodermatitis		Photoexacerbated diseases		Dermatitis with dryness		Other diseases		All diseases	
	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD
Erythema										
Before	34	1.7 \pm 1.1	35	2.1 \pm 1.1	53	1.2 \pm 1.2	7	1.6 \pm 0.8	129	1.6 \pm 1.2
After		1.0 \pm 1.0		1.5 \pm 1.1		0.8 \pm 0.9		1.1 \pm 0.4		1.0 \pm 1.0
P		<.001**		<.001**		<.001**		.180		<.001**
Itching										
Before	34	1.3 \pm 1.2	35	1.3 \pm 1.4	53	1.2 \pm 1.1	7	1.0 \pm 1.2	129	1.3 \pm 1.2
After		0.7 \pm 0.8		0.7 \pm 0.9		0.8 \pm 0.9		0.6 \pm 0.8		0.7 \pm 0.9
P		<.001**		<.001**		<.001**		.180		<.001**
Dryness										
Before	33	1.4 \pm 1.2	35	1.2 \pm 1.0	53	1.5 \pm 1.0	6	1.2 \pm 1.0	127	1.4 \pm 1.1
After		0.8 \pm 1.0		0.6 \pm 0.7		0.9 \pm 0.9		0.7 \pm 0.5		0.8 \pm 0.9
P		.001**		<.001**		<.001**		.180		<.001**

Note: P-value was calculated using the Wilcoxon signed-rank test.

** $P < .01$.

TABLE 3 Changes in bioactive substances from stratum corneum analysis by application of sunscreen

	Photodermatosis		Photoexacerbated diseases		Dermatitis with dryness		Other diseases		All diseases	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
IL-1ra/IL-1α [-]										
Before	28	103.735 ± 156.394	33	74.122 ± 86.903	52	105.775 ± 164.509	6	34.554 ± 39.729	119	92.926 ± 140.645
After		73.532 ± 128.969		64.002 ± 95.755		66.746 ± 138.531		41.490 ± 31.231		66.308 ± 121.133
P		.019*		.469		.181		1.000		.016*
TNF-α [-]										
Before	28	5.95 ± 1.90	35	4.55 ± 1.22	52	5.79 ± 2.20	6	4.79 ± 1.59	121	5.42 ± 1.94
After		5.49 ± 1.78		5.05 ± 1.97		5.21 ± 1.73		4.96 ± 1.34		5.22 ± 1.78
P		.038*		.055		.006**		.600		.040*
Bax [-]										
Before	28	6.41 ± 1.89	35	6.48 ± 2.36	52	4.01 ± 1.75	6	5.55 ± 1.52	121	5.36 ± 2.28
After		5.62 ± 1.48		6.45 ± 2.30		3.98 ± 1.86		5.42 ± 1.65		5.14 ± 2.17
P		.003**		.539		.012*		.917		.005**
TLR3 [-]										
Before	18	40.35 ± 20.01	23	31.88 ± 15.16	41	57.14 ± 47.72	5	34.68 ± 27.71	87	45.70 ± 36.87
After		56.81 ± 29.48		37.68 ± 20.90		87.85 ± 48.72		46.08 ± 29.16		65.76 ± 43.70
P		.016*		.191		<.001**		.686		<.001**

Note: P-value was calculated using the Wilcoxon signed-rank test.

*P < .05.

**P < .01.

we predicted that its level would be reduced after sunscreen use. Indeed, in healthy individuals, TLR3 levels were significantly lower in unexposed body parts relative to that in sun-exposed body parts. However, TLR3 levels were significantly elevated in photodermatosis patients, counter to what was predicted. Another study reported that interferon-gamma enhances TLR3 expression in keratinocytes,²³ suggesting that other factors besides UV exposure are involved. Further investigation is required to validate TLR3 as an early indicator of the influence of sun exposure on the skin.

In conclusion, changes in the expression of IL-1ra/IL-1 α , TNF- α , and Bax were linked to sunscreen use and also reflected the changes in skin symptoms after sunscreen protection, suggesting that they may serve as early indicators of the influence of sun exposure on the skin. The use of these indicators should assist in the early detection of sun exposure effects on the skin and thus help patients with diseases like photodermatosis in their daily life, preventing chronic damage.

ACKNOWLEDGEMENTS

We thank Dr Hiroshi Shimizu (Department of Dermatology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University) and Dr Hironobu Ihn (Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University) for their contributions to patient recruitment and acquisition of data. However, Dr Hiroshi Shimizu passed away on February 17, 2021, and Dr Hironobu Ihn passed away on March 13, 2020. We thank them both for their guidance.

CONFLICT OF INTEREST

Tokiwa Pharmaceutical Co., Ltd. funded this clinical trial and the test products.

AUTHOR CONTRIBUTION

Atsushi Fukunaga, Chikako Nishigori, and Hiroshi Matsunaka involved in conception and design of the study. Atsushi Fukunaga, Satoshi Fukushima, Hiroaki Iwata, Makiko Nakahara, Rikako Sasaki, Naoko Baba, Masutaka Furue, and Chikako Nishigori involved in patient recruitment and acquisition of data. Hiroshi Matsunaka and Yumi Murakami analyzed the data. Atsushi Fukunaga drafted the manuscript. Chikako Nishigori revised the manuscript critically for important intellectual content. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Atsushi Fukunaga  <https://orcid.org/0000-0003-2026-8154>

Satoshi Fukushima  <https://orcid.org/0000-0002-0622-7682>

Makiko Nakahara  <https://orcid.org/0000-0002-4169-1309>

Hiroshi Matsunaka  <https://orcid.org/0000-0002-9635-8132>

Yumi Murakami  <https://orcid.org/0000-0003-3540-9140>

Masutaka Furue  <https://orcid.org/0000-0002-2967-1073>

REFERENCES

- Miyamura Y, Coelho SG, Wolber R, et al. Regulation of human skin pigmentation and responses to ultraviolet radiation. *Pigment Cell Res.* 2007;20:2-13.
- Fisher GJ, Wang Z, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *New Eng J Med.* 1997;337:1419-1429.
- Imokawa G. Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. *Pigment Cell Res.* 2004;17:96-110.
- Flament F, Bazin R, Laquieze S, Rubert V, Simonpietri E, Piot B. Effect of the sun on visible clinical signs of aging in Caucasian skin. *Clin Cosmet Invest Dermatol.* 2013;6:221.
- Nishigori C. Cellular aspects of photocarcinogenesis. *Photochem Photobiol Sci.* 2006;5:208-214.
- Barr RM, Walker SL, Tsang W, et al. Suppressed alloantigen presentation, increased TNF-alpha, IL-1, IL-1Ra, IL-10, and modulation of TNF-R in UV-irradiated human skin. *J Invest Dermatol.* 1999;112:692-698.
- Ying G-S, Maguire M, Quinn G, Kulp MT, Cyert L. ROC analysis of the accuracy of noncycloplegic retinoscopy, retinomax autorefractor, and suresight vision screener for preschool vision screening. *Investig Ophthalmol vis Sci.* 2011;52:9658-9664.
- Bernard JJ, Cowing-Zitron C, Nakatsuji T, et al. Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. *Nat Med.* 2012;18:1286-1290.
- Mittal D, Saccheri F, Vénéreau E, Pusterla T, Bianchi ME, Rescigno M. TLR4-mediated skin carcinogenesis is dependent on immune and radioresistant cells. *EMBO J.* 2010;29:2242-2252.
- O'Grady A, Kay EW, McKenna DB, Bennett MA, Murphy GM, Leader MB. Altered expression of the p53-regulated proteins, p21Waf1/Cip1, MDM2, and Bax in ultraviolet-irradiated human skin. *Hum Pathol.* 1998;29:559-564.
- Gozali MV, Zhou B-R, Luo D. Update on treatment of photodermatosis. *Dermatol Online J.* 2016;22(2):268-286.
- Moriwaki S, Kanda F, Hayashi M, Yamashita D, Sakai Y, Nishigori C. Xeroderma pigmentosum clinical practice guidelines. *J Dermatol.* 2017;44:1087-1096.
- Kondoh M, Ueda M, Nakagawa K, Ichihashi M. Siblings with xeroderma pigmentosum complementation group A with different skin cancer development: importance of sun protection at an early age. *J Am Acad Dermatol.* 1994;31:993-996.
- Stern RS, Weinstein MC, Baker SG. Risk reduction for non-melanoma skin cancer with childhood sunscreen use. *Arch Dermatol.* 1986;122:537-545.
- Young AR, Claveau J, Rossi AB. Ultraviolet radiation and the skin: photobiology and sunscreen photoprotection. *J Am Acad Dermatol.* 2017;76:S100-S109.
- Hughes MCB, Williams GM, Baker P, Green AC. Sunscreen and prevention of skin aging: a randomized trial. *Ann Intern Med.* 2013;158:781-790.
- Boyd AS, Naylor M, Cameron GS, Pearse AD, Gaskell SA, Neldner KH. The effects of chronic sunscreen use on the histologic changes of dermatoheliosis. *J Am Acad Dermatol.* 1995;33:941-946.
- Fukunaga A, Nishigori C, Ito K, et al. Evaluation of the effectiveness of sunscreens for photosensitive disorders. *Nishinohon J Dermatol.* 2011;73:271-277.

19. Terui T, Hirao T, Sato Y, et al. An increased ratio of interleukin-1 receptor antagonist to interleukin-1 α in inflammatory skin diseases. *Exp Dermatol*. 1998;7:327-334.
20. Morita E, Takahashi H, Niihara H, et al. Stratum corneum TARC level is a new indicator of lesional skin inflammation in atopic dermatitis. *Allergy*. 2010;65:1166-1172.
21. Hirao T, Aoki H, Yoshida T, Sato Y, Kamoda H. Elevation of interleukin 1 receptor antagonist in the stratum corneum of sun-exposed and ultraviolet B-irradiated human skin. *J Invest Dermatol*. 1996;106:1102-1107.
22. Bashir M, Sharma M, Werth V. TNF- α production in the skin. *Arch Dermatol Res*. 2009;301:87-91.
23. Kajita A, Morizane S, Takiguchi T, Yamamoto T, Yamada M, Iwatsuki K. Interferon-gamma enhances TLR3 expression and anti-viral activity in Keratinocytes. *J Invest Dermatol*. 2015;135:2005-2011.

How to cite this article: Fukunaga A, Fukushima S, Iwata H, et al. Bioactive substances in the stratum corneum of the epidermis found as indicators of skin damage due to sun exposure. *Photodermatol Photoimmunol Photomed*. 2022;38:241-249. <https://doi.org/10.1111/phpp.12743>