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Addition of UVA-absorber butyl methoxy dibenzoylmethane to topical ketoprofen formulation reduces ketoprofen-photoallergic reaction

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ABSTRACT

Topical application of ketoprofen (KP) clinically evokes the allergic type of photocontact dermatitis. To avoid this adverse reaction, we investigated the beneficial effect of each ultraviolet (UV) filter that was included in topical ketoprofen formulation. We first tested the inhibitory effects of four UVA filters by a modified local lymph node assay following KP application on the mouse skin and UVA irradiation on the same site. In this assessment, butyl methoxy dibenzoylmethane (BMDBM), when included in KP application, exerted the most effective inhibitory effect on stimulation with KP and UVA. We manufactured topical patch and gel KP applicants containing BMDBM, which retained KP penetration through the skin and KP stability toward UVA. The ability of BMDBM in these formulations to inhibit KP photosensitivity was evaluated by a modified adjuvant and strip method in guinea pigs, and the photoallergic reactions induced by the BMDBM-containing KP applicants were lower than the non-containing ones. It is known that KP has a cross-reactivity with benzophenone upon UVA exposure, but such a photocross-reactivity of BMDBM with KP was not observed in a mouse ear swelling model. The anti-inflammatory effect of the BMDBM-containing KP patch applicant was comparable to the non-containing one. These results suggest that the addition of BMDBM into KP topical formulations is efficacious for inhibition of KP photocontact dermatitis.

1. Introduction

Various topical agents, such as antibacterial agents, perfumes, sunscreens, and non-steroidal anti-inflammatory drugs (NSAIDs), have been reported to cause photocontact dermatitis [1]. This sensitivity is evoked when these agents are topically applied to the skin and the same site was subsequently exposed to ultraviolet (UV) light. The photoreactions are divided into the phototoxic and photoallergic types. While the phototoxic reaction is mediated by oxygen intermediates without specific immune reactions [2,3], photoallergic reaction occurs as a consequence of a specific immune reaction mediated by antigen-specific, sensitized T cells [4–8]. Two mechanisms have been put forward in T-cell recognition of photoallergic small molecules as photoantigen, i.e. photohapten and prohapten theories [1,5,8]. According to the photohapten theory, photosensitizing chemicals and protein need to coexist, and upon exposure to UV, the chemicals bind covalently to protein. On the other hand, the prohapten theory suggests that UV simply converts photosensitizing substances into ordinary hapten, which subsequently binds to protein.

Ketoprofen (KP) is widely used as a topical NSAID, since its transdermal penetration and pharmacological efficiency are superior to other NSAIDs such as diclofenac and indomethacin [9]. Whereas orally administered NSAIDs may cause gastrointestinal and cardiovascular adverse reactions, the skin application of NSAIDs very rarely induces these systemic side effects. However, topical formulations of KP may provoke photocontact dermatitis as an adverse reaction. KP has both phototoxic and photoallergic potentials, but many clinical observations have indicated that photosensitivity to KP is a photoallergic reaction with action spectrum of UVA [8]. KP serves as a photohapten because of its photocoupling ability to protein. Our previous studies have shown that KP application plus UVA irradiation induces and elicits photocontact dermatitis in mice, and both $CD4^+$ and $CD8^+$ T cells are required for the full-blown sensitivity reaction [10]. In addition, KP plus UVA upregulates the expression of MHC class II and costimulatory molecules on murine dendritic cells, promoting antigen-presenting ability of the cells [11]. Dendritic cells bearing KP-photoantigen sensitize photoantigen-specific T cells in draining lymph node cells [10].

Avoidance of UVA exposure to the skin site applied with KP prevents photocontact dermatitis. The use of sunscreen UV filters is

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one of the common methods for UV protection. In Japan, the patch application is the most popular formulation when KP is topically administered. Employment of UVA filters added to the topical formulations is one of the efficacious strategies to prevent contact photosensitivity. UVA filters can be classified into chemical UVA filters (UV absorbers) or physical UVA filters (UV-scattering nanoparticles). Since UV absorbers convert UV light into the thermal energy, some UV absorbers are photolabile and may have a photosensitive potential. Moreover, it is well known that UVA absorber oxybenzone (benzophenone-3, OX) is photocross-reactive with KP [12–14].

In this study, we investigated the preventive effect of UV filters on KP contact photosensitivity. We first selected an effective UVA filter and tested its ability to suppress the photosensitivity by adding it into the KP patch and gel formulations. Results suggest that the addition of the UVA absorber to topical KP formulations is effective to reduce the photosensitivity without cross-reactivity.

2. Materials and methods

2.1. Animals

Female BALB/c mice (8–10 weeks old) and female Hartley guinea pigs (6 weeks old) were obtained from SLC, Inc. (Hamamatsu, Japan). Female hairless mice (8 weeks old) were obtained from Kudo Co., Ltd. (Tosu, Japan). Male Lewis rats (6 weeks old) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). All animals were maintained in our animal facility, exposed to a 12 h light: 12 h dark cycle, and provided with food and water *ad libitum*. All animal experiments were performed with the approval of the Laboratory Animal Care and Use Committee at Hisamitsu Pharmaceutical Co., according to Laboratory Animal Welfare guidelines.

2.2. Reagents and topical KP formulations

KP, butyl methoxy dibenzoylmethane (BMDBM) and OX were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Diethylamino hydroxybenzoyl hexyl benzoate (DHHB) was purchased from BASF Japan Ltd. (Tokyo, Japan). Hydrophobic particle (mean size: 20 × 100 nm) of titanium dioxide (TiO_2) was purchased from Sakai Chemical Industry Co., Ltd. (Sakai, Japan). All other chemicals were reagent grade or better.

We manufactured two formulations of KP transdermal patch application, i.e. a patch containing BMDBM (BMDBM (+) patch) and a patch non-containing BMDBM (BMDBM (−) patch), by a hot melt method. Both BMDBM (+) and BMDBM (−) patches contained 2% KP. In BMDBM (+) patch, its adhesive layer contained 2% BMDBM, while no UVA filter was added to BMDBM (−) patch. The adhesive layer of both patches consisted of styrene isoprene styrene block copolymer and polyisobutylene, and was laminated on weaved fabric backing. Thickness of the adhesive layer and the fabric backing was approximately 100 and 600 μm, respectively. The other side was covered with a release liner film until use. In addition to the patch application, we also used the gel application, which was Ketum (BMDBM (−) gel) purchased from Menarini France (Rungis, France). To prepare BMDBM-containing KP gel (BMDBM (+) gel), we added BMDBM to Ketum at 1% final concentration and mixed them extensively before use.

2.3. Light source

As UVA source, 40-W black light (FL40SBLB-A) emitting UVA ranging from 320 to 400 nm with a peak emission at 365 nm was purchased from Toshiba Electric Co. (Tokyo, Japan). With a UV radiometer (Topcon Technohouse Corp., Tokyo, Japan), the energy output of eight black light tubes at a distance of 20 cm was 3 mW/cm^2 at 310–400 nm. Irradiation was performed through a pane of 3 mm thickness glass.

2.4. Modified local lymph node assay

Local lymph node assay (LLNA) was performed, as described previously [15], with some modifications to examine photosensitivity. Mice were painted on the dorsal aspect of each earlobe with 25 μL of 2% KP alone or 2% KP plus various concentrations of BMDBM, OX, DHHB or TiO_2 in acetone/olive oil (4:1), and subsequently irradiated with 20 J/cm^2 UVA. The painting plus irradiation was performed on three consecutive days, i.e., days 0, 1 and 2. On day 5, all mice were injected intravenously with 250 μL of phosphate buffered saline (PBS) containing 20 μCi [methyl-^{3}H] thymidine (^{3}H-TdR). Five hours later, the mice were sacrificed, and the draining auricular lymph nodes were removed and pooled for each experimental group. Single cell suspensions of lymph node cells (LNC) were prepared by gentle mechanical disaggregation through cell strainer using the plunger of a syringe. LNC were centrifuged at 1100 rpm for 10 min, washed with 3 mL of PBS and resuspended with 3 mL of 5% trichloroacetic acid (TCA). After overnight incubation at 4 °C, the precipitate was recovered by centrifugation, resuspended with 1 mL of 5% TCA, and transferred to 10 mL of scintillation fluid. ^{3}H-TdR incorporation was measured by β-scintillation counter (Hitachi Aloka Medical, Ltd., Tokyo, Japan). Percentage of control was calculated according to the following formula:

$$\%\ \text{of control} = (\text{sample} - \text{vehicle control})/(\text{KP alone control} - \text{vehicle control}) \times 100.$$

2.5. In vitro skin penetration assay for KP

Penetration of KP through the skin was evaluated by using the Franz diffusion cell system. The diameter of the diffusion cell system used in this study was 2.5 cm, which corresponded to an effective permeable area of 4.9 cm^2. In the mouse skin system, the dorsal skin was excised from sacrificed hairless mice and its fat tissue was removed. In the human skin system, full-thickness human skin samples were obtained from Human and Animal Bridging Research Organization (Tokyo, Japan). After removal of the subcutaneous fat, the skin containing stratum corneum was subsequently sliced using an electric dermatome to 500 μm-thickness materials. Each topical KP formulation was applied to mouse or human skin, and the skin was immediately mounted on the Franz diffusion cell. The receptor chamber was filled with PBS, stirred by a magnetic bar, and maintained at 32 °C. The receptor fluid was flowed at approximately 5 mL/h and collected by an auto-sampler. A part of the collected receptor fluid was mixed with the same volume of acetonitrile. The mixture was centrifuged for 5 min, and the supernatant was transferred into a glass vial. Quantitative analysis of KP was performed on a HPLC Shimadzu-XR (Shimadzu Corp., Kyoto, Japan) equipped with UV detector (254 nm) using a TSK-gel ODS-80Ts column (Tosoh Corp., Tokyo, Japan). The HPLC analysis was performed as follows: the mobile phase, 0.1% acetic acid/acetonitrile; flow rate, 1:1; and injection volume, 20 μL.

2.6. Quantitation of KP and BMDBM in earlobes

Mice were painted with each KP patch formulation on both sides of earlobes for 4 h. After removal of the KP patch, mice were sacrificed, and the earlobes were immediately excised and minced

with surgical scissors. These minces were ground to fine powder by a frost shattering system under liquid nitrogen freezing. The specimens of ground earlobes were placed into a tube at 20 mg. As extraction solvent, 2 mL of methanol was added into the tubes, and the tubes were shaken for 15 min. After centrifugation at 3000 rpm for 10 min, supernatants were transferred to a glass vial. Quantitative analysis of KP was performed as described above. BMDBM was also quantified on a HPLC Shimadzu-XR equipped with UV detector (358 nm) using a TSK-gel ODS-80Ts column. The analysis was performed as follows: mobile phase, 0.1% acetic acid/acetonitrile; flow rate, gradient ratio from 1:1 to 1:20 (1 mL/min), and injection volume, 20 μL.

2.7. KP photostability in topical formulations

After removal of the release liner film, the adhesive aspect of KP patch formulation was irradiated with varying doses of UVA. Residual KP contained in the UVA-irradiated patch was then extracted with tetrahydofuran, and the extracts were diluted with methanol up to a final volume of 50 mL. For the gel formulations, each KP gel was spread on a PET film at 11.4 mg/cm^2 and irradiated with varying doses of UVA. Residual KP contained in the UVA-irradiated gel was extracted with water/methanol (1:1), and the extracts were diluted with methanol up to 50 mL. A part of each extract was filtrated with a pretreatment disk for HPLC (Tosoh Corp.) and transferred into a glass vial. Intact KP was quantified by HPLC as described above.

2.8. Photosensitization and photochallenge to KP in guinea pigs

In our preliminary study, photosensitivity to KP was not induced or elicited in guinea pigs by topical application of KP formulations and subsequent UVA irradiation without adjuvant injection. However, when the adjuvant and strip method as described previously [16] with some modifications was used, KP photosensitivity was detected. By using this method, we investigated the inhibitory effect of BMDBM addition on KP photoallergy. On day 0, guinea pigs were injected subcutaneously with 0.1 mL of emulsified mixture of Freund's complete adjuvant (Wako Pure Chemical Industries, Ltd.) and saline (1:1) at four corners of the application site on the clipped neck (8 cm^2). Each KP patch formulation (8 cm^2) or each KP gel (90 mg) was applied on the neck for 4 h and irradiated with 10 J/cm^2 UVA. The KP application and UVA irradiation to the tape-stripped skin was performed once daily for five consecutive days. In the photosensitization to both KP patch and gel, there were three kinds of application, such as non-application (Group A and B), BMDBM (−) (Group C and D) and BMDBM (+) (Group E and F). On day 21, the guinea pigs were photochallenged with each KP patch (4 cm^2) or KP gel (45 mg) applied for 4 h and subsequent UVA at 10 J/cm^2 on the clipped dorsal skin. In the photochallenge, two skin sites were used for BMDBM (−) (Group A, C and E) and BMDBM (+) (Group B, D and F) formulations. The skin reactions on the photochallenged sites were graded at 24 and 48 h after UVA irradiation according to Draize grading criteria [17]. Erythema and eschar formation are as follows: Score 0, no erythema; Score 1, very slight erythema; Score 2, well-defined erythema; Score 3, moderate to severe erythema; and Score 4, severe erythema to slight eschar formation. Edema formation was as follows: Score 0, no edema; Score 1, very slight edema; Score 2, slight edema; Score 3, moderate edema; and Score 4, severe edema.

2.9. Photosensitization and photochallenge to KP in mice

The mouse model of photocontact dermatitis was described previously [10,18]. Mice were painted with 50 μL of 8% KP, 10% OX or 10% BMDBM in acetone/olive oil (4:1) to the clipped dorsal skin and subsequently irradiated with 40 J/cm^2 of UVA. The painting plus irradiation was performed once daily for three consecutive days. Before photochallenge, the basal line thickness of both ears on all mice was measured with a dial thickness gauge. On day 5, all mice were challenged on both sides of each earlobe with 25 μL of 2% KP, 10% OX or 10% BMDBM in acetone/olive oil (4:1) and subsequently irradiated with 40 J/cm^2 UVA. Ear thickness was measured 24 h after irradiation and was expressed as the mean increment in thickness above basal line control value.

2.10. Chronic inflammatory model

The anti-inflammatory activity of the KP patch formulations was evaluated by using an adjuvant-induced arthritic model [19]. Lewis rats were injected with 0.1 mL of 1% *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI) in liquid paraffin into the left hind paw. On day 14 after the adjuvant injection, each KP patch formulation (1 cm^2) was topically applied to the right hind paw for 24 h, and the application was performed for 7 days. The volume of right hind paw was measured on day 1, 4 and 7 after the first application with a plethysmometer (MK-101CMP; Muromachi Kikai Co., Ltd., Tokyo, Japan). Simultaneously, the edema rate was calculated according to the following formula:

$$\text{Edema rate } (\%) = [(\text{paw volume after adjuvant injection}) / (\text{paw volume before adjuvant injection}) - 1] \times 100.$$

2.11. Statistical analysis

Statistical analysis was performed by Student's *t*-test or Dunnett type multiple comparison using SAS software (SAS Institute Japan Ltd., Tokyo, Japan). *P*-values less than 0.05 were considered to be significant.

3. Results

3.1. Selection of BMDBM as KP-photoprotective agent

In advance of manufacturing UV filter-containing, topical KP formulations, we first compared the potentials of four sunscreen agents to inhibit KP photosensitivity, as assessed by modified LLNA. BMDBM and DHHB are commonly used UVA absorbers, OX is a conventional UV absorber, and TiO_2 is a nanoparticle UV filter. The radio-uptake of draining lymph node cells was measured. Data were expressed as the percentage of control in KP-augmented ^{3}H-TdR incorporation (Fig. 1). The inhibitory effect of BMDBM was strongest among the four filters, especially at a low concentration of 0.25%. Therefore, we chose BMDBM as an UV-protective reagent for topical KP formulations.

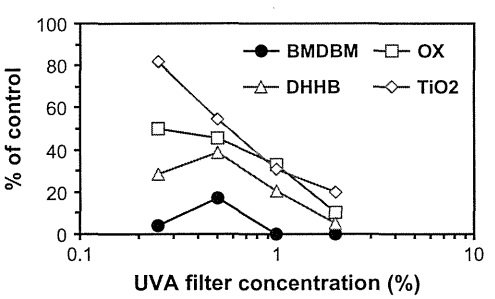

Fig. 1. Inhibitory effects of various UVA filters in organic solvent for KP photosensitivity assessed by murine lymph node proliferation assay.

3.2. No disturbance of KP penetration by BMDBM addition in patch or gel formulation

We examined the skin permeability of KP included in the patch and gel formulations that contained BMDBM [BMDBM (+)] or not [BMDBM (−)]. KP was quantified with HPLC. The cumulative amounts of KP at 12 h in BMDBM (+) patch, BMDBM (−) patch, BMDBM (+) gel, and BMDBM (−) gel were 45.8, 38.5, 15.9 and 14.7 μg/cm^2, respectively (Fig. 2A), without significant differences between BMDBM (+) and BMDBM (−).

To confirm the KP permeability in human skin, each topical KP formulation was applied to the human skin mounted on the Franz diffusion cells, and flowed fluid samples collected by an auto-sampler were quantified with HPLC. Again, the cumulative amount of KP was not significantly different between BMDBM (+) and BMDBM (−) patch formulations (Fig. 2B).

In another experiment, mice were painted with each of the KP patch formulations on both sides of earlobes for 4 h. The earlobes were excised and minced to fine powder by a frost shattering system. Quantitative analyses of KP and BMDBM were performed with HPLC. There was no significant difference in the KP amount between BMDBM (+) and BMDBM (−) patch formulations, while BMDBM was detected in BMDBM (+) but not BMDBM (−) patch (Fig. 3).

3.3. Prevention of photodegradation of KP by BMDBM

The KP photostability in the patch and gel BMDBM (+) formulations irradiated with various amounts of UVA was examined. KP-containing materials were extracted from the irradiated patch and gel formulations, and each extract was quantified for intact KP amount by HPLC. UVA irradiation induced photodegradation of KP in a dose-dependent manner (Fig. 4). The amounts of intact KP in the BMDBM (+) patch and gel were significantly higher than in the BMDBM (−) ones (Fig. 4). Upon exposure to 40 J/cm^2 UVA, intact KP amount was reduced to 26.9% in the BMDBM (−) patch, however, 91.6% of KP remained in the BMDBM (+) patch. Thus, BMDBM effectively protected KP from UVA exposure.

3.4. Suppression of KP photosensitivity by BMDBM in guinea pigs

Guinea pigs were photosensitized and photochallenged with KP patch (Table 1, upper panel) or gel (Table 1, lower panel) formulations and subsequent UVA irradiation. BMDBM was added or not in each KP formulation. Upon challenge, UVA was required for the positive skin reaction (data not shown). In the non-sensitized animals, neither photochallenge with the BMDBM (−) nor BMDBM (+) KP formulation induced responses (Groups A and B). When photosensitized with the BMDBM (−) KP formulation (patch or gel), photochallenge with the BMDBM (−) formulation evoked skin responses (Group C). The gel formulation (lower panel) was more effective than the patch formulation for induction of the sensitivity. However, the BMDBM (+) formulation yielded weaker responses (Group D) than the BMDBM (−) one (Group C). The photosensitized guinea pigs with the BMDBM (+) formulation exhibited weak responses upon challenge with the BMDBM (−) formulation (Group E), but did not show substantial responses with the BMDBM (+) formulation (Group F). Thus, the results indicated that BMDBM effectively depressed the both sensitization and challenge of KP photosensitivity in the guinea pig model.

3.5. No photocross-reaction between KP and BMDBM

It is known that KP photocross-reacts with OX [12–14]. To test the possible cross-reactivity between KP and BMDBM, we used a mouse model of photocontact dermatitis. Mice were photosensitized and photochallenged with KP or OX (Fig. 5A) or with KP or BMDBM (Fig. 5B). Significant ear swelling responses were obtained in KP-photosensitized and photochallenged mice. In this study, as compared to the vehicle sensitization, neither OX (Fig. 5A) nor BMDBM (Fig. 5B) induced significant levels of the photosensitivity. In the KP-photosensitized mice, however, photochallenge with OX elicited a significant response to a lesser degree than photochallenge with KP, suggesting photocross reactivity between KP and OX. On the other hand, photocross reactivity between KP and BMDBM was not observed, as represented by no significant ear

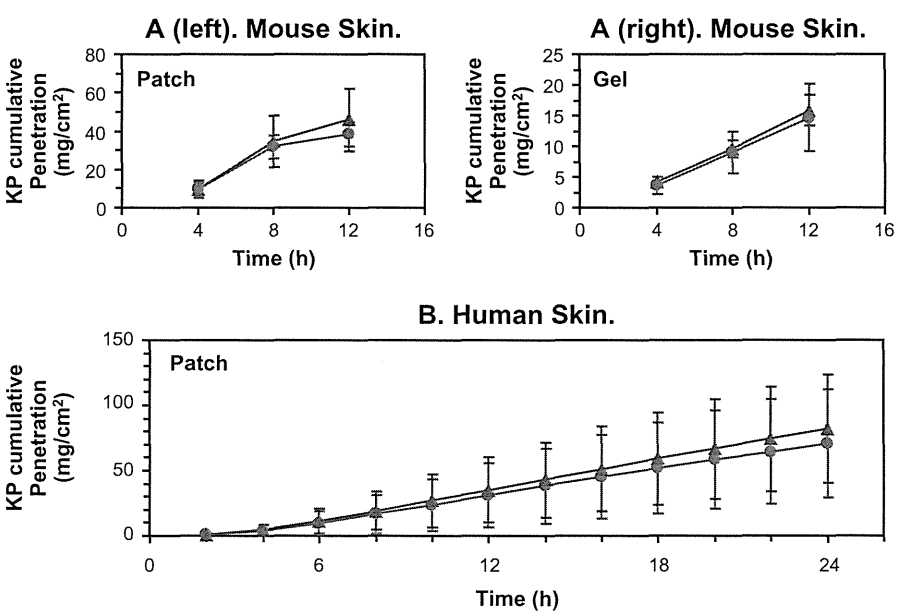

Fig. 2. Effects of BMDBM on KP penetration in topical KP formulations. (A) Data indicate the mean values of KP flux through the mouse skin applied with BMDBM (−) formulation (red circle) or BMDBM (+) formulation (blue triangle). The error bars represent S.D. ($n = 3$). (B) Data indicate the mean values of cumulative KP penetration through the human skin applied with BMDBM (−) patch (red circle) or BMDBM (+) patch (blue triangle). The error bars represent S.D. ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

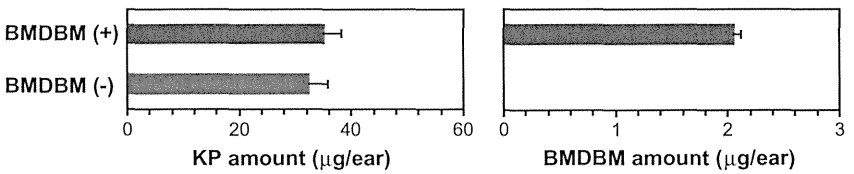

Fig. 3. KP and BMDBM absorption on murine ear from patch formulations. Data indicate the mean values of KP and BMDBM amounts in mouse ear applied with BMDBM (−) patch (red column) or BMDBM (+) patch (blue column). The error bars represent S.D. ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

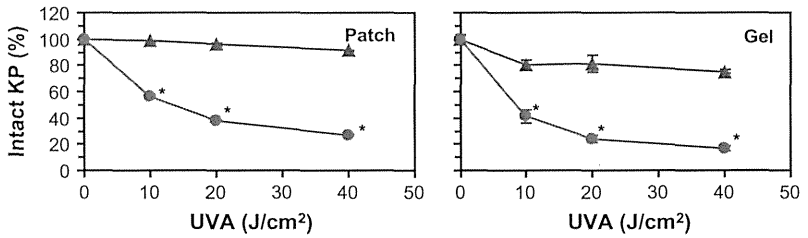

Fig. 4. Inhibitory effect of BMDBM for KP photostability in topical KP formulations. Data indicate the mean values of intact KP percentage in BMDBM (−) formulation (red circle) or BMDBM (+) formulation (blue triangle). The error bars represent S.D. ($n = 3$). Statistical analysis was carried out with Student's *t*-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

swelling upon photochallenge with BMDBM in KP-photosensitized mice (Fig. 5B).

3.6. No abrogation of the anti-inflammatory ability of KP in BMDBM formulation

We finally compared the anti-inflammatory ability between the BMDBM (+) and BMDBM (−) patch formulations using an adjuvant-induced arthritis rat model. Seven days after beginning of KP application (day 21 after adjuvant injection), the edema rate of hind paw was 95.7% in the positive control without KP (Fig. 6). When applied with the BMDBM (+) and BMDBM (−) formulations, the edema rates were comparably decreased to 60.4% and 50.7%, respectively. This suggested that the anti-inflammatory ability of KP was not abrogated by BMDBM addition.

4. Discussion

Although UVA is the action spectrum of photosensitivity to most of exogenous agents such as KP [8], there are not many available UV filters capable of filtrating UVA wave range. We demonstrated that BMDBM had the strongest potential to reduce KP photosensitivity among the four sunscreen agents tested. In addition, BMDBM (+) patch indicated the similar skin penetration kinetics of KP to BMDBM (−) patch in two different skin studies. Therefore we considered BMDBM was the best UV filter to reduce the photosensitivity to KP patch. It is known that UV absorbers, such as OX, paradoxically can evoke photocontact dermatitis [20–22]. In this respect, UV reflector, as exemplified by TiO_2, is more photostable and less likely to have its photoallergy than UVA absorbers. Although the inhibitory ability of TiO_2 was weaker than BMDBM, we also manufactured a KP patch formulation containing TiO_2. However, the inhibitory effect of TiO_2 addition was not observed in the animal model (data not shown). Since TiO_2 in organic solvent reduced the KP photosensitivity, TiO_2 might be useful as topical formulations such as gel, lotion and cream.

In the present study, significant ear swelling responses of mice photosensitized and photochallenged with 10% BMDBM or 10% OX was not found, while 2% KP significantly induced the photoallergic reactions. Therefore, the photosensitive potentials of BMDBM and OX are greatly lower than that of KP. It has been reported that photocross-reactions occur between KP and other chemicals, such as OX, harbouring a benzophenone moiety [12–14]. We showed that BMDBM was not photocross-reactive with KP in the mouse ear swelling model. In addition, we found using adjuvant and strip method that BMDBM was not photocross-reactivity with KP, while OX cross-reacted with KP (data not shown). From the viewpoint of cross-reactivity, it is also considered that BMDBM is one of the best choices as UVA filter added to KP patch formulations.

KP undergoes a decarboxylation process upon irradiation with UVA in an aqueous solution. Several groups have reported that the main photoproduct of KP formed under an aerobic solution is (3-benzoylphenyl) ethane [23,24]. Photodecarboxylation of KP to (3-benzoylphenyl) ethane proceeds two pathways *via* benzylic carbanion and benzylic radical intermediates [23,25,26]. These radical products from KP might induce covalent binding of KP with protein, when they coexist with each other. Therefore, improvement of KP photostability possibly reduces KP photoallergy. In our study, KP photostability in the topical formulations was significantly elevated by BMDBM addition. It is thought that BMDBM reduces UVA-induced KP decarboxylation and the following formation of radical intermediates.

Table 1
Inhibitory effect of BMDBM in topical KP formulations for KP photosensitivity in guinea pigs.

Group	Photosensitization	Photochallenge	*N*	Mean score	
				24 h	48 h
A	–	BMDBM (−) patch	6	0.0	0.0
B	–	BMDBM (+) patch	6	0.0	0.0
C	BMDBM (−) patch	BMDBM (−) patch	6	1.0	1.2
D	BMDBM (−) patch	BMDBM (+) patch	6	0.7	1.0
E	BMDBM (+) patch	BMDBM (−) patch	6	0.2	0.3
F	BMDBM (+) patch	BMDBM (+) patch	6	0.0	0.3
A	–	BMDBM (−) gel	6	0.0	0.0
B	–	BMDBM (+) gel	6	0.0	0.0
C	BMDBM (−) gel	BMDBM (−) gel	6	3.2	3.5
D	BMDBM (−) gel	BMDBM (+) gel	6	2.0	2.3
E	BMDBM (+) gel	BMDBM (−) gel	6	1.0	1.8
F	BMDBM (+) gel	BMDBM (+) gel	6	0.3	0.7

Mean score indicates an average of total skin reactions graded according to Draize grading criteria [(score of erythema and eschar formation) + (score of edema)].

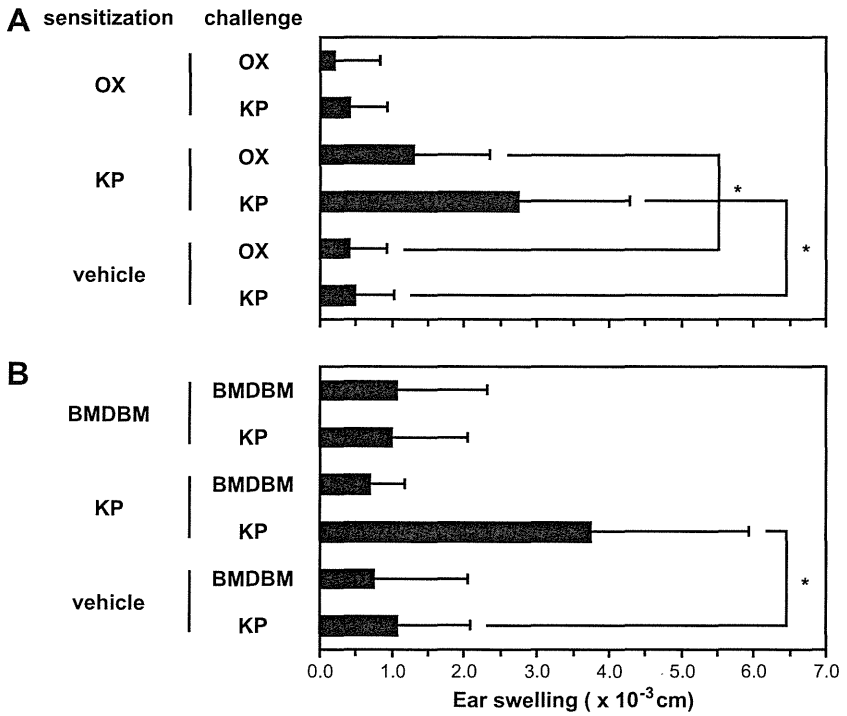

Fig. 5. Cross reactivity of BMDBM to KP in mouse ear swelling test. Mice were photosensitized with 8% KP, 10% OX or 10% BMDBM in acetone/olive oil (4:1) on the clipped dorsal skin. Data indicate mean values of expression as the mean increment in thickness above basal line control value. The error bars represent S.D. (n = 5). Statistical analysis was carried out with Student's t-test.

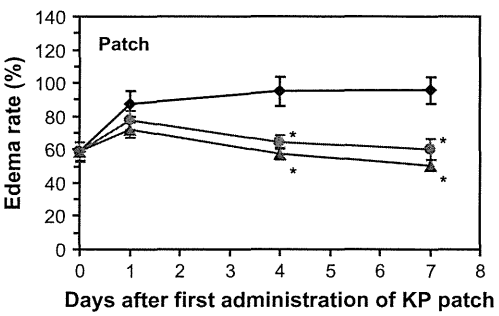

Fig. 6. Anti-inflammatory effect of KP patch formulation containing BMDBM in the adjuvant-induced arthritic model. Data indicate the mean values of edema rate in the rat paw applied with control patch without KP (black diamond), BMDBM (−) patch (red circle) or BMDBM (+) patch (blue triangle). The error bars represent S.E. (n = 12). Statistical analysis was carried out with Dunnett type multiple comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Phototreatment with KP induces DNA damage and peroxidation of cell membranes because of radical derivatives and active oxygens [26–28]. The damage reactions to cells cause not only phototoxicity but also photoallergy where immune cells in the skin, such as dendritic cells and keratinocytes, are involved. KP phototreatment upregulates the antigen presenting ability of dendritic cells and promotes the production of cytokines in the epidermis [11]. The photoallergic sensitivity is divided into the photosensitization and the photoelicitation phases. In our mouse KP photocontact dermatitis model [10], we have shown that both KP application and UVA irradiation are mandatory for the sensitivity. In the present guinea pig model, the addition of BMDBM into either sensitization or elicitation formulation successfully reduced the degree of the sensitivity. However, the patients who have already been sensitized with KP should not use even the sunscreen-containing topical formulation. Moreover, although the absorption of BMDBM from patch was fewer than that of KP, in the modified LLNA, the lymph node cell proliferation augmented by KP patch plus UVA was significantly decreased by BMDBM addition (data not shown). In addition, the inhibitory effect of BMDBM addition on KP phototoxicity was also found in mouse ear swelling model (data not shown). Since BMDBM was effective even at a low amount, BMDBM may sufficiently absorb the UV active wavelengths to induce the photosensitivity.

In conclusion, our study demonstrated that BMDBM in topical KP formulations photostabilizes KP and may reduce the photoallergic adverse reaction in clinical use. Photocross-reactivity of BMDBM with KP seems to be absent. The addition of BMDBM does not affect KP permeability and anti-inflammatory activity.

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Paediatric Acute Generalized Exanthematous Pustulosis Induced by Paracetamol with High Serum Levels of Interleukin-8 and -22: A Case Report

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Acute generalized exanthematous pustulosis (AGEP) is a rare skin disorder, characterized by acute development of numerous, pin-head sized, non-follicular, sterile pustules that usually begin in intertriginous folds with high fever and neutrophilia (1–3). The condition is frequently induced by hypersensitivity reaction to drugs (1). AGEP usually affects adults; paediatric cases have rarely been reported (4–7). We describe here a case of paediatric AGEP induced by paracetamol. High levels of serum interleukin (IL)-8 and IL-22 observed in our patient suggest a role of these cytokines/chemokines in the pathogenesis of AGEP.

CASE REPORT

A 7-year-old boy was referred to us with a generalized eruption. Five days prior to our initial examination, the patient had developed upper respiratory symptoms, diagnosed as influenza A, and he had received oral paracetamol and oseltamivir. Two days after the start of treatment, he developed an itchy exanthema on the trunk with a high fever. On examination, the patient had an erythematous eruption on his trunk (Fig. 1a) and the proximal parts of his arms and thighs. Numerous small pustules, less than 1 mm in diameter, were present, especially on the inner aspects of the thighs (Fig. 1b), axillae, and lumbar region. Slightly swollen cervical lymph nodes were palpable. Laboratory investigations showed a normal leukocyte count, but C-reactive protein was elevated (1.4 mg/dl; normal < 0.1 mg/dl).

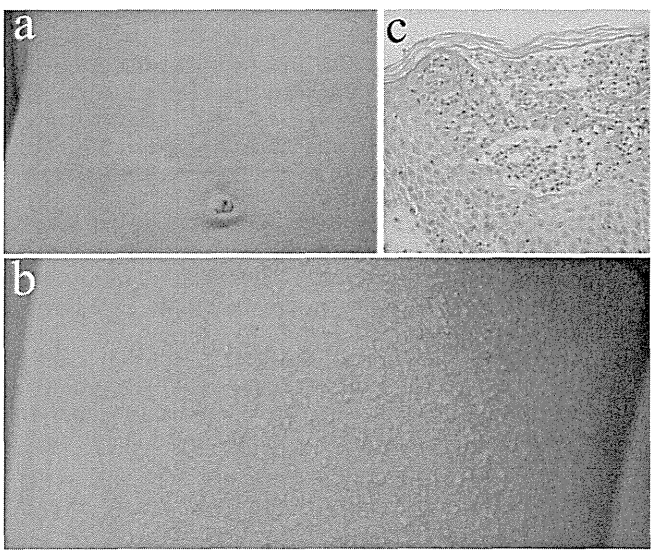

Fig. 1. Clinical and histopathological appearances. (a) Clinical appearance, showing an erythematous eruption present on the trunk. (b) Close-up view, showing multiple small pustules on the thigh. (c) Histopathology, exhibiting subcorneal collection of many neutrophils in the epidermis (haematoxylin and eosin (HE), original magnification ×200).

Histopathologically, there were subcorneal neutrophilic pustules and a dermal lymphocytic infiltrate (Fig. 1c). We determined the likelihood of AGEP by using the reported scoring system, which can be used to identify cases of AGEP based on morphology, course and histology of the skin reaction (8). Our patient had a score of 11, indicating a definite diagnosis of AGEP. The culprit drugs and results of lymphocyte transformation test performed 19 days after disease onset were as follows (stimulation index [SI] ≥ 1.8 is considered positive): paracetamol, 1,516 cpm (SI 2.75); oseltamivir, 450 cpm (SI 0.81); and no-addition control, 551 cpm. We thus diagnosed the eruption as AGEP induced by paracetamol. Discontinuation of paracetamol and oral administration of prednisolone (5 mg daily for 7 days) improved the patient's skin lesions within 2 weeks.

The serum level of IL-8 was measured with Cytometric Bead Array (BD Biosciences, San Diego, CA, USA), serum levels of IL-17A, IL-22 and TNF-α with enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), and that of chemerin with an ELISA kit (Millipore, St Charles, MO, USA) in the patient and 4 healthy individuals. These cytokines and chemokines may be involved in the accumulation of neutrophils in the epidermis (9, 10). A blood sample was taken from the patient 5 days after the onset of eruption. It is noted that IL-8 and IL-22 were markedly elevated in the patient, compared with the normal individuals (Table I). IL-17 and tumour necrosis factor-α (TNF-α) were below the level of detection in both patient and normal healthy controls. Chemerin, a chemoattractant for plasmacytoid dendritic cells (pDC) (11, 12), was not increased in the patient compared with the normal subjects.

DISCUSSION

AGEP should be differentiated from pustular psoriasis (von-Zumbusch type). In our patient, the skin eruption and high fever were improved by discontinuation of paracetamol and 7-day administration of prednisolone (5 mg daily), and there was no recurrence thereafter. This shorter duration supports the diagnosis of AGEP. The SI of 2.75 in a lymphocyte stimulation test was significantly high (13). The possibility of the causative role of infection could not be completely ruled out, but at least paracetamol contributed to the eruption,

Table I. *Serum levels of cytokines/chemokines*

	Serum levels	
Cytokines/chemokines	Patient	Normal subjects (n=4, mean ± SD)
IL-8	274.6 pg/ml	1.29 ± 1.49 pg/ml
IL-17A	UDL	UDL (all)
IL-22	25.3 pg/ml	UDL (all)
TNF-α	UDL	UDL (all)
Chemerin	176.8 pg/ml	229.0 ± 31.0 pg/ml

UDL: under the detection level; SD: standard deviation.

and infection may participate in the occurrence of the eruption. Although AGEP is generally considered to be an adult disease (1–3), recent reports suggest that it occasionally occurs in paediatric individuals (4–7). Thus, children may develop AGEP upon administration of antibiotics or non-steroidal anti-inflammatory drugs, as seen in our patient.

As the lymphocyte transformation test with a causative drug usually shows a high SI, drug-specific T cells are thought to mediate AGEP (2, 3). Drug-specific $CD4^+$ and $CD8^+$ T cells play an important role by producing neutrophil chemo-attractant IL-8. To explain the mechanism of subcorneal accumulation of neutrophils, however, a certain population of drug-specific T cells are thought to stimulate keratinocytes to produce IL-8, and the keratinocyte-derived IL-8 may contribute to the accumulation of neutrophils in the lesional epidermis. In fact, the elevated expression of IL-8 was observed in keratinocytes as well as infiltrating mononuclear cells (3).

Th17 cell is a $CD4^+$ T helper cell subset capable of producing IL-17 and IL-22, and dysregulated Th17 responses mediate a variety of skin inflammatory conditions, such as psoriasis (9) and atopic dermatitis (14). IL-17 and IL-22 exert a strong synergistic effect on the production of IL-8 by keratinocytes (14). Increased frequencies of Th17 cells and high levels of IL-22 have been reported in AGEP (15, 16).

Our study showed an increase in serum IL-8 and IL-22 in a paediatric patient with AGEP. Since the amount of IL-17A was below the limit of detection, the involvement of Th17 cells remains unclear in this single case report. In order to maintain Th17 cells, IL-23 released from dendritic cells (DCs) is important (10), and DCs are activated by TNF-α in an autocrine manner (9). Alternatively, type I interferon derived from pDCs may indirectly lead to Th17 cell stimulation with the help of chemerin serving as a pDC-chemo-attracting factor (11). While patients with psoriasis have higher levels of chemerin (12), our AGEP patient did not have an increased level of chemerin in the peripheral blood, suggesting that pDC are not substantially involved in the pathogenesis. Although IL-17 and/or IL-22 may be involved in the pathogenesis of AGEP, the exact role of Th17 cells in this drug eruption, and their stimulation mechanism, are as yet unknown.

The authors declare no conflicts of interest.

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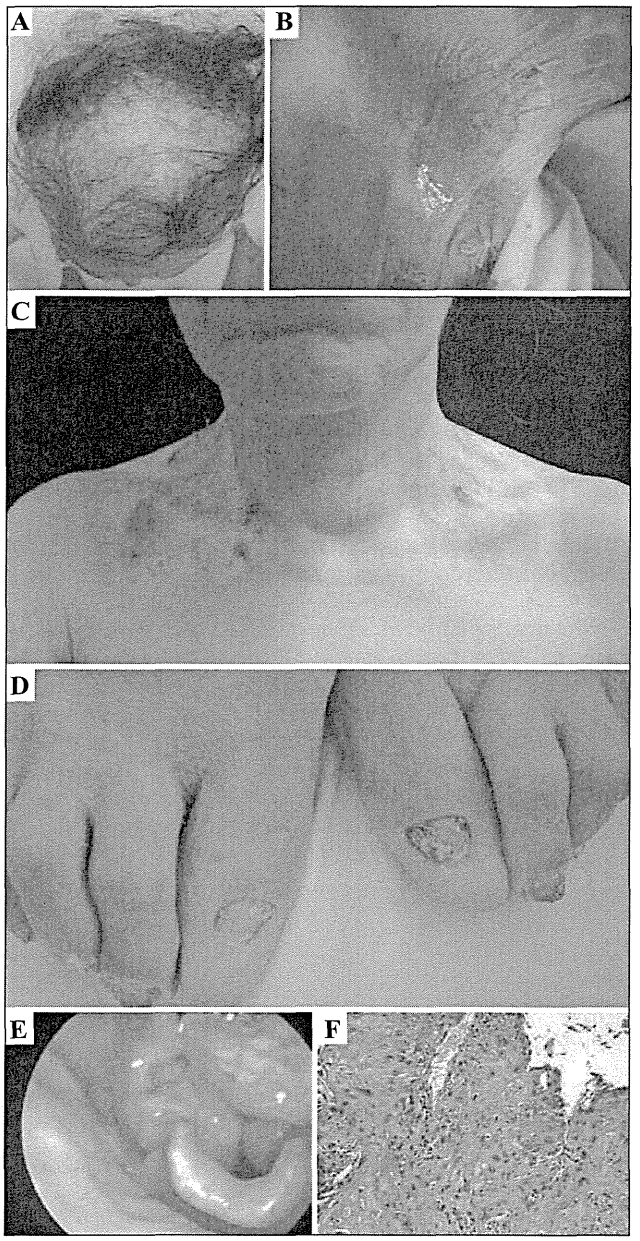

Figure 1. A) Cicatricial alopecia on the scalp; **B)** Erosions, bulla and atrophic, hypopigmented scars in the axillary region; **C)** Atrophic, hypopigmented scars at the neck; **D)** Dystrophic changes in the nails; **E)** Endoscopic image of the tumor; **F)** Atypical cell groups consistant with squamous cell carcinoma (hematoxylin-eosin stain, magnification ×100).

cutaneous lesions [4]. The etiology of esophageal carcinoma is related to exposure of the esophageal mucosa to noxious or toxic stimuli. Smoking and chronic alcohol exposures are the most common etiological factors for SCC. Vitamin and nutritional defects have been recognized as contributing factors. The epithelial lining of the esophagus in junctional EB and DEB is subject to chronic inflammation and damage. This may increase the risk of epithelial metaplasia and malignancy. Additionally, esophageal strictures cause vitamin, iron, and nutritional deficiencies in DEB. Recently, abnormalities in the p53 and p16 tumor suppressor genes in RDEB-associated SCC have been demonstrated. All these factors could play roles in the development of esophageal SCC in DEB [6].

Because of the serious complications of EB, patients with dysphagia should be followed by a multidisciplinary team for life-threatening risks. ■

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Drug eruption due to sodium picosulfate

A 62-year-old man who had been suffering from severe constipation presented with a 2-day history of pruritic papules and vesicules on the trunk and extremities. He had been prescribed sodium picosulfate, sennoside, pantethine and magnesium oxide sporadically over the course of 10 years. Medical examination revealed tense vesicles and blisters on the extremities and palms *(figures 1A-B)*. Vital signs were within normal ranges. There were no symptoms of fever, lymphadenopathy, mucous or systemic involvement throughout the disease course. The laboratory result was negative for herpes simplex virus antibodies, varicella zoster virus IgG was 3.6 (normal range <2.0), and Epstein-Barr virus antibodies indicated previous infection.

A skin biopsy from a tense blister on the left arm revealed a subepidermal blister with eosinophilic infiltration *(figures 1C-D)*. Direct immunofluorescence labeling of the lesional skin sections showed no IgG, IgM, IgA or C3 deposition at the basement membrane zone. Circulating anti-BP180 autoantibody was negative by ELISA. Antinuclear antibody was negative. Patch tests were negative for all the medicines prescribed (see above). A lymphocyte stimulation test (LST) was positive for sodium picosulfate twice; the stimulation index was 208% (normal <180%) at Day 5 and 187% at Day 33, however this LST was negative with three healthy controls. In contrast, sennoside, pantethine and magnesium oxide were all negative at Days 5 and 33. After discontinuation of all laxatives including sodium picosulfate, the patient's eruptions subsided remarkably without therapy. Thus, the case was diagnosed as sodium picosulfate-induced bullous eruption. The patient has recently taken all the laxatives except for sodium picosulfate, but he has not suffered from a drug eruption.

Sodium picosulfate is a popular laxative. It is not digested in the stomach or the small intestine; it is hydrolysated in the large intestine and transformed to active diphenole compounds. These active compounds stimulate intestinal motility and prevent water absorption, resulting in relief from constipation. Most sodium picosulfate compounds are excreted in the feces. When the usual dosage is taken, only a tiny portion is absorbed, and this is glucuronidated in the liver and excreted in urine and bile. Thus, in general, the side effects of sodium picosulfate are limited to abdominal pain and nausea.

To our knowledge, there has only been one other report of sodium picosulfate-induced drug eruptions: a fixed drug eruption reported in the Japanese literature [1]. The eruptions in that case appeared after 5 months of sodium picosulfate intake. Our patient had a ten-year history of sodium picosulfate intake. These cases suggest that long-term intake of sodium picosulfate can induce eruptions.

The pathomechanism of such eruptions is uncertain. Sennoside, another laxative, is also scarcely absorbed in the intestine, similar to sodium picosulfate, and sennoside has been reported to lead to drug eruptions after long-term intake [2-4]. We presume that drug eruptions induced by laxatives are caused by delayed T-cell hypersensitivity, which might explain why the skin eruptions occur after long-term intake. For drug eruptions to develop, it might take a long time for T cells to become sensitized or for the drug or reactive metabolites to achieve sufficient distribution. Further studies are needed to fully understand the mechanisms.

In conclusion, sodium picosulfate is generally believed to be safe, because allergic reactions are so rare. Thus, patients tend to self-medicate with it frequently and persistently for constipation. However, the present case suggests that we should be aware that sodium picosulfate can induce drug eruptions after long-term intake. ■

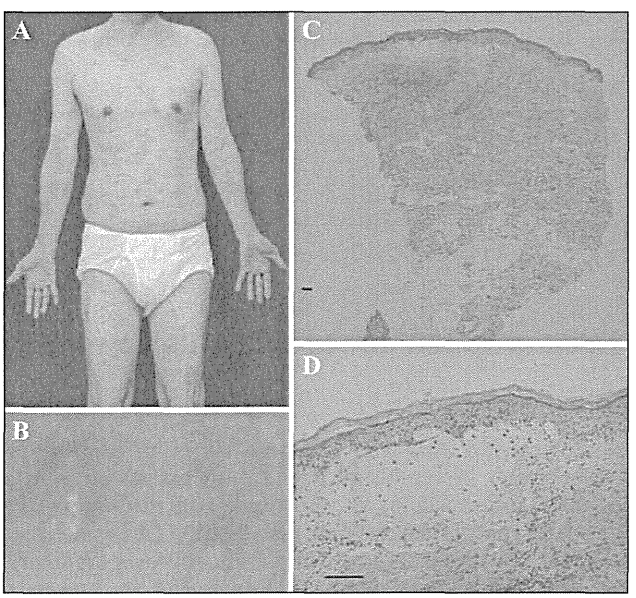

Figure 1. Skin manifestations and pathological tissue of the patient.
A) Widespread papulo-vesicular eruption in a 62-year-old man. **B)** Vesicles on the left arm. **C)** Skin biopsy specimen from the left arm showing subepidermal blister formation. **D)** There is mixed eosinophilic and lymphocytic infiltration in the bulla. (C-D: Hematoxylin and eosin stain; Bar=100 μm).

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The syndrome of inappropriate antidiuretic hormone secretion (SIADH) associated with metastatic malignant melanoma

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a state where excess vasopressin is secreted from hypophysis in conditions when sufficient water is sustained in the body [1]. Although SIADH occurs in

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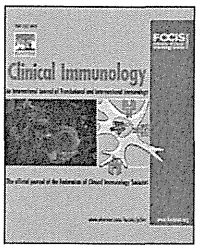

LETTER TO THE EDITOR

Disturbed balance in three subpopulations of CD4$^+$Foxp3$^+$ regulatory T cells in Stevens–Johnson syndrome and toxic epidermal necrolysis patients

KEYWORDS
Stevens–Johnson syndrome (SJS);
Toxic epidermal necrolysis (TEN);
Regulatory T cell;
Treg-subpopulations

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening diseases that are two of the most severe cutaneous adverse reactions [1]. The regulatory T cell (Treg) has been reported to be involved in the pathogenesis of SJS/TEN [2,3]. Treg maintains self-tolerance and suppresses immune responses. It was reported that even though the relative frequency of Treg is normal in TEN, Treg function is profoundly impaired, which may result in epidermal damage by excessive activation of effector T cells [3]. Recent reports have shown that CD4$^+$Foxp3$^+$ Treg exists in heterogeneous subpopulations divided by the level of CD45RA expression [4]. Human CD4$^+$Foxp3$^+$ Treg is divisible into three functionally and phenotypically distinct subsets: CD4$^+$CD45RA$^+$ Foxp3^{low} resting Treg (rTreg), CD4$^+$CD45RA$^-$Foxp3^{high} activated Treg (aTreg) and cytokine-secreting CD4$^+$CD45RA$^-$Foxp3^{low} nonsuppressive T cells (non-Treg). rTreg and aTreg have potently immunosuppressive activity, whereas non-Treg lacks such activity and has the potential to secrete proinflammatory cytokines such as IFN-γ and IL-17 [4]. Furthermore, the relative frequencies of the three CD4$^+$Foxp3$^+$ Treg subpopulations differ with various disease conditions [5–7]: in active systemic lupus erythematosus, for example, the number of aTreg cells is lower and that of non-Treg cells is higher than those of healthy controls, allowing an autoimmune reaction to develop [4]. SJS/TEN might also be considered susceptible to development under the imbalance of CD4$^+$Foxp3$^+$ Treg subpopulations. This study investigates the relative frequencies and cytokine production characteristics of CD4$^+$Foxp3$^+$ Treg subpopulations in SJS/TEN patients.

The investigations were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patients' parents. All control subjects gave informed consent in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMCs) from SJS/TEN patients (acute stage: n = 3, resolution stage: n = 7) (Supplemental Table 1) and 24 healthy controls were prepared by Ficoll gradient centrifugation. PBMCs were stained with anti-CD4, anti-CD45RA and anti-Foxp3, and then analyzed by flow cytometry (materials and methods are detailed in Supplemental data). The three subpopulations of Treg were defined by the expression of CD45RA and Foxp3 (Fig. 1a). In these three subpopulations, the frequency of non-Treg, which has no suppressive potential, in the acute stage of SJS/TEN (2.04 ± 0.89%) was significantly lower, but that in the resolution stage of SJS/TEN (4.50 ± 0.87%) was significantly higher than that of the healthy controls (3.61 ± 0.87%) (Fig. 1b). In contrast, rTreg and aTreg did not differ among these groups. Over 95% of CD4$^+$CD25$^+$Foxp3$^+$ cells showed low CD127 expression (Supplemental Fig. 1). From this data, in our experiments, CD4$^+$CD25$^+$Foxp3$^+$ cells were defined as regulatory T cells.

Furthermore, the three CD4$^+$Foxp3$^+$ Treg subpopulations were analyzed for the expression of cytokines in patients in the resolution stage of SJS/TEN by intracellular cytokine staining assay. Interestingly, the percentage of IFN-γ-producing aTreg in patients in the resolution stage of SJS/TEN was significantly lower than that of healthy controls, whereas IFN-γ-secreting non-Treg and rTreg showed no differences between SJS/TEN patients and healthy controls. There were no significant differences in frequency of IL-17A-producing cells among the three Treg subpopulations (Supplemental Table 2).

The present study shows that the frequency of non-Treg in the acute stage of SJS/TEN was significantly lower, but that the frequency of non-Treg in the resolution stage of SJS/TEN was significantly higher than that of healthy controls. Among Treg subpopulations, only the frequency of non-Treg drastically changed during the disease course. Although it is not known why the relative frequency of non-Treg is equal before and after disease, the relative frequency in the resolution stage of SJS/TEN is higher than that of healthy controls, indicating that non-Treg might influence susceptibility to SJS/TEN.

Furthermore, the frequency of IFN-γ-producing aTreg in SJS/TEN patients was significantly lower than that of the healthy controls. With respect to plasticity, Treg was found to differentiate into helper T cells and to express T-bet, which is a master transcription factor of Th1 effector cells which produce IFN-γ. T-bet$^+$Foxp3$^+$ Treg inhibits the Th1-dependent response [8]. We demonstrated that the frequency of IFN-γ-secreting aTreg cells, namely Th1-like aTreg, was significantly lower in SJS/TEN patients than in healthy controls. There are several

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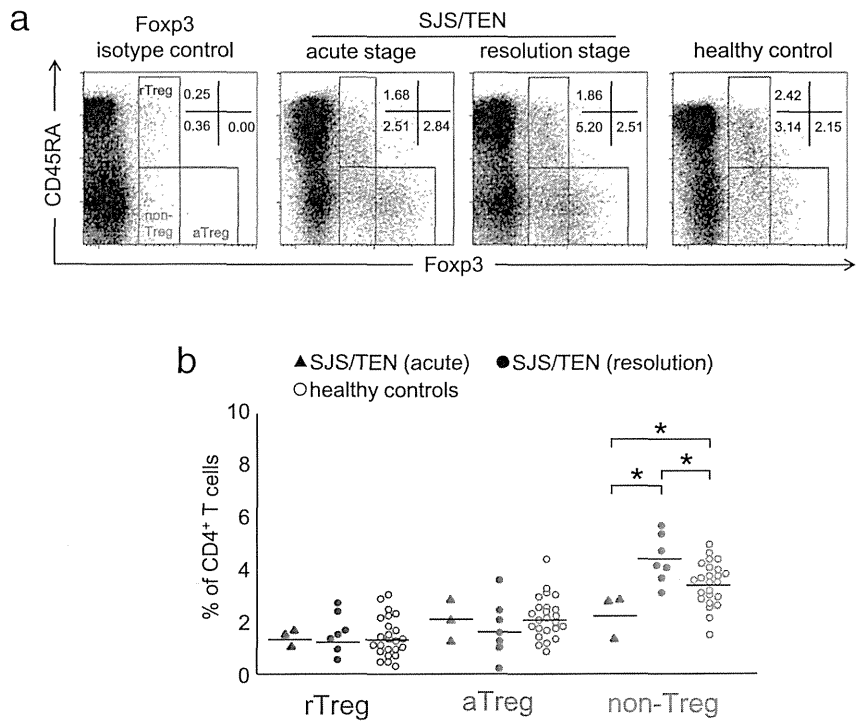

Figure 1 The relative frequencies and cytokine production characteristics of the three $CD4^+Foxp3^+$ Treg subpopulations in patients who had recovered from SJS/TEN and in healthy controls. (a) Three subpopulations of $CD4^+$ T cells defined by the expression of CD45RA and Foxp3: $CD45RA^+Foxp3^{low}$ rTreg, $CD45RA^-Foxp3^{high}$ aTreg, $CD45RA^-Foxp3^{low}$ non-Treg. The percentage of each area in CD45RA and Foxp3 is shown. (b) Percentages of each $Foxp3^+$ subpopulation among $CD4^+$ T cells in patients with SJS/TEN (open circles, n = 7) and healthy controls (closed circles, n = 24) (*; $p < 0.05$; NS: not significant).

reports about Th1/Th2 polarization in SJS/TEN. The SJS/TEN lesions showed a mixed Th1/Th2 pattern [9]. Quaglino P., et al. showed the coexistence of a Th2 response in addition to the predominant Th1 profile in blood [10]. In addition, Th2 cells are known to outnumber Th1 cells. On the other hand, the percentage of T-bet$^+$ was 56.7% in aTreg. IFN-γ production is an indirect indication of Th1-controlling Treg cells [8]. Our results suggest that the Th1-like aTreg subpopulation in SJS/TEN patients reduces the inhibitory capacity under immune active conditions, such as drug-mediated allergic reaction.

To date, no reports have identified a correlation between IL-17 production of $CD4^+$ T cells (Th17) and SJS/TEN development. In our study, we also investigated the frequency of Th17. That frequency did not differ significantly between SJS/TEN patients and healthy controls (SJS/TEN; 1.92 ± 1.01%, healthy controls; 1.80 ± 0.72%, P = 0.80).

In conclusion, the relative frequencies of the three Treg subpopulations and cytokine-secreting activity are found to differ between SJS/TEN patients and healthy controls. These results indicate that the disturbed balance of Treg subpopulations is involved in the pathogenesis of SJS/TEN.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clim.2013.04.002.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

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30 March 2013

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Laminins, which are prominent components of basement membranes, play important roles for organ development and function, including skin, hair, skeletal muscle, nervous system, kidney, lung and vasculature. Laminins are large heterotrimeric glycoproteins composed of one α-, one β- and one γ-chain. There are currently five α-, four β- and three γ-chain genes that have been described in vertebrates and the chains can assemble into at least 15 different heterotrimers. The molecular specificity of anti-laminin autoantibodies generally determines the phenotype of autoimmune subepidermal blistering disease. In patients with anti-laminin-γ1 pemphigoid, subepidermal blisters with neutrophil infiltration, which are predominant skin lesions, heal rapidly in response to treatment without scarring resolution.[3] The exact laminin targeted by anti-laminin-γ1 antibodies has not yet been identified. On the other hand, a chronic course and debilitating scarring of multiple mucous surfaces are considered to be the common clinical features of most patients with anti-laminin-332 mucous membrane pemphigoid. Previously, two patients with an autoimmune response to both laminin-γ1 and laminin-332 were reported.[1,4] In these cases, tense blisters affecting both skin and mucous membranes, which were characteristics of two distinct clinical phenotypes in anti-laminin-γ1 pemphigoid and anti-laminin-332 mucous membrane pemphigoid, were observed. Our case presented with flaccid blisters on the dorsal surface of her body, while mucous membranes were not affected. This is not typical for both anti-laminin-γ1 pemphigoid and anti-laminin-332 mucous membrane pemphigoid. It is possible that both anti-laminin-γ1 and anti-laminin-332 autoantibodies synergistically induced this unusual clinical phenotype. It is also possible that in pemphigoid patients without mucosal involvement showing anti-laminin-332 autoantibodies, unrevealed antibodies triggered the development of skin pemphigoid, which was followed by the epitope-spreading phenomenon of autoantibodies against laminin-332.[5] Interestingly, as in our patient, although the α3- and γ1-subunits in laminin-6 (α3β1γ1) and laminin-7 (α3β2γ1) are detectable in alveolar basement membranes, no respiratory complications were reported in anti-laminin-γ1 pemphigoid and anti-laminin-332 mucous membrane pemphigoid.

Although in contrast to laminin-332, the pathogenicity of anti-laminin-γ1 autoantibodies could not been shown, we should take into consideration the possibility of dual detection of autoantibodies against laminin-γ1 and laminin-332 in patients with subepidermal blistering disease. A higher number of patients with combined autoimmunity against laminin-γ1 and -α3 is needed to further define the clinical phenotype in this variant.

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Adult case of Stevens–Johnson syndrome possibly induced by *Chlamydophila pneumoniae* infection with severe involvement of bronchial epithelium resulting in constructive respiratory disorder

Dear Editor,

A 48-year-old man developed high fever and conjunctivitis 2 days after taking a supplementary drink, then taking loxoprofen sodium hydrate subsequently. On that night, he developed generalized macropapular eruptions, ulcerations with hemorrhagic crusting of the lip and oral mucosa (Fig. 1a,b), bilateral bulbar conjunctivitis and partial corneal ulceration, dyspnea with increasing amount of sputum and was admitted to our hospital. There were no obvious findings in chest roentgenogram.

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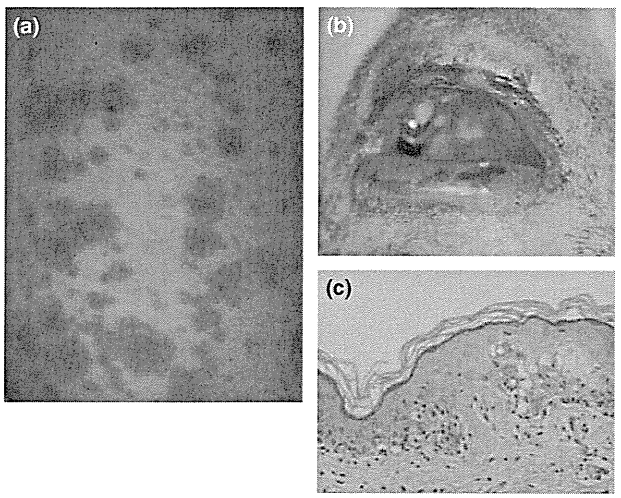

Figure 1. Clinical appearances and histopathological findings on admission. (a) Macropapular eruptions with atypical targetoid lesions were observed in the whole body. (b) Ulcerations with hemorrhagic crusting of the lip and oral mucosa were also observed. (c) Hematoxylin–eosin-stained section of the biopsy specimen showed the liquefaction degeneration and segmental necrosis of keratinocytes in the basal layer (original magnification ×200).

According to histological findings of skin biopsy (Fig. 1c) and clinical features, we diagnosed as Stevens–Johnson syndrome (SJS) with 9% of detached body surface area. In spite of immediate steroid pulse therapy, his dyspnea progressed and artificial ventilation was performed. Bronchoscopy revealed almost total loss of bronchial epithelium (Fig. 2a). Mucosal involvement due to SJS was still expanding and a series of plasmapheresis and subsequent of human i.v. immunoglobulin (Ig) administrations were performed. His respiratory status was gradually resolved together with skin and mucosal re-epithelization after these treatments. Chest computed tomography on day 39 showed bronchiectasis and cavitation including abscess (Fig. 2b). Pulmonary function tests on day 65 were consistent with restrictive defect.

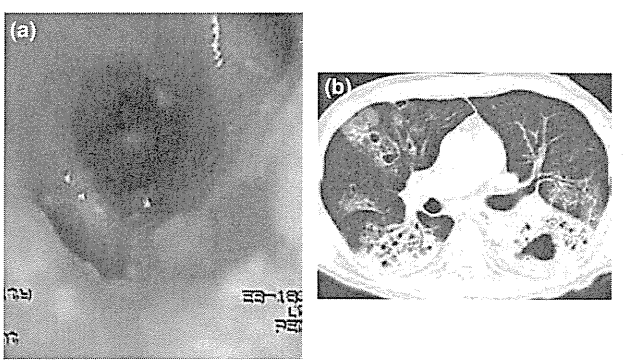

Figure 2. Imaging analysis for the respiratory involvement. (a) Bronchoscopy showed severe involvement of bronchial epithelium. (b) Chest computed tomography showed obvious consolidation, ground-glass appearance with bronchiectasis and cavitation including abscess predominately in the dorsal area.

His conjunctivitis had already appeared before taking loxoprofen sodium hydrate and the results of drug-induced lymphocyte stimulation test (DLST) on admission day was negative. DLST for supplementary drink was also negative. Serological investigation for viruses (herpes simplex, herpes zoster, Epstein–Barr, cytomegalovirus) and *Mycoplasma pneumoniae* DNA by polymerase chain reaction (PCR) were negative. Reactivation of human herpesvirus 6 was also denied. However, an elevated *Chlamydophila pneumoniae* immunoglobulin IgG (enzyme-linked immunoassay; day 1:0.06 [–], day 12:1.17 [+], day 18:2.01 [+]) was detected. Then, we considered that it might be a case of SJS related to *C. pneumoniae* infection rather than other infection or medication.

Stevens–Johnson syndrome and toxic epidermal necrolysis (TEN) are considered to be variations of the same disease and complications in various organs in each disease have not always been discussed separately. Pulmonary complications in TEN were reported to indicate poor prognosis,[1] while in SJS, they are common in cases related to *M. pneumoniae*, but lead a mild clinical course[2] without severe late sequelae disorder in respiratory function. *M. pneumoniae*-associated SJS affects mainly children and most adult cases are caused by medications. From these investigations, massive structural and functional damage of the lungs in adult SJS like our case is thought to be rare compared to in TEN patients.

We considered the possibility that our case was SJS associated with *C. pneumoniae* infection. However, the third serological test might have detected transfused IgG by plasma exchange and i.v. immunoglobulin. We should have performed further examination such as PCR analysis of the sputum for definite diagnosis for *C. pneumoniae*. *M. pneumoniae* and *C. pneumoniae* infection show similar clinical manifestations and the former are common among younger patients and the latter among older patients.[3] There has been no published work describing the relation between *C. pneumoniae* infection and SJS, however, there may be some cases regarded as SJS rerated to *M. pneumoniae* infection or idiopathy, however, that have actually been caused by *C. pneumoniae* infection.

Stevens–Johnson syndrome patients with pulmonary involvement have been reported to show dyspnea and bronchial hypersecretion as primary symptoms without obvious findings by roentgenogram, while bronchoscopy revealed bronchial involvement during the early clinical period which correlated with poor prognosis.[4] Bronchoscopy may be useful for cases with the primary symptoms described above to estimate the severity.

Pulmonary complications of SJS/TEN are recognized as interstitial pneumonia in the acute phase and obliterative bronchitis in the chronic phase. During the acute phase, standard treatments for SJS like our case are usually applied. However, once the obliterative bronchitis has been established, lung transplantation is thought to be the only curative therapy.[5] Careful follow up is required for patients with pulmonary complications.

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Hydrolyzed wheat protein-containing facial soap-induced wheat-dependent exercise-induced anaphylaxis in a patient without filaggrin mutations

Dear Editor,

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a type of immediate hypersensitivity in which anaphylaxis develops when exercise is performed within several hours of ingestion of wheat products. Recently, an increasing number of patients with WDEIA who developed the condition during or after using continuously hydrolyzed wheat protein (HWP)-containing soap have been reported in Japan;[1] however, not all users of HWP (Glupearl 19S)-containing soap suffer from WDEIA. Therefore, its predisposing factor should be pursued.

Filaggrin (*FLG*) is an epidermal protein that optimizes and maintains skin barrier functions. A deficiency in *FLG* is a major predisposing factor to the development of atopic dermatitis, contact hypersensitivity to nickel, and other allergic diseases, such as peanut allergy.[2] This raises the notion that a skin barrier defect plays an important role in WDEIA associated with HWP. Herein, we report a patient with WDEIA sensitized to HWP that was used in facial soap without filaggrin mutations.

A 51-year-old woman had been using HWP-containing facial soap frequently and vigorously every day since 2004. Three months after she started to use HWP-containing soap, she suddenly developed angioedema on the eyelids and an urticarial rash on the face. She experienced similar episodes many times between 2004 and 2009 when eating food containing wheat was followed by mild exercise, but she kept using HWP-containing facial soap continuously. Her symptoms were limited to her face. In July 2009, she developed anaphylactic shock after ingesting normal wheat products. Based on the anaphylactic shock episode, we suspected that she had WDEIA. She had no history of atopic dermatitis or gastrointestinal food hypersensitivities. In addition, she did not exhibit dry skin.

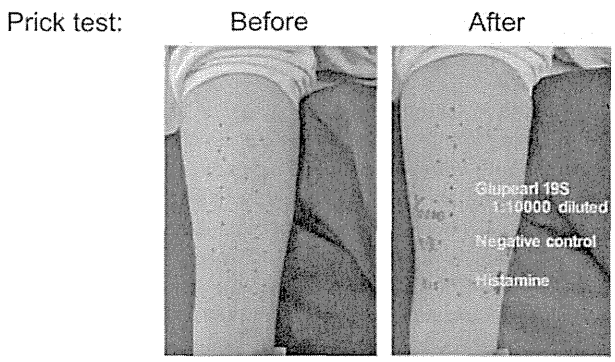

Figure 1. Skin prick test with Glupearl 19S diluted 1:10 000 induced severe generalized urticarial rash on the entire upper extremity.

After a skin prick test with Glupearl 19S diluted 1:10 000, she developed eyelid angioedema, dyspnea and a generalized urticarial rash on her entire upper extremity (Fig. 1). A serum allergen-specific immunoglobulin (Ig)E test (Immuno CAP; Phadia, Uppsala, Sweden) for wheat was 0.36 UA/mL and gluten-specific IgE was 0.40 UA/mL. Serum omega-5 gliadin-specific IgE antibody titers were within normal limits. Most patients with a common type of WDEIA reacted to omega-5 gliadin, but patients with HWP-WDEIA did not have specific IgE for omega-5 gliadin.

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皮膚病診療：35(12)；1169～1172, 2013

◇薬疹——2013<臨床例>——⑬

アスピリンが原因薬と考えられた中毒性表皮壊死症

山賀　康右*　花房　崇明*　山岡　俊文*　小豆澤宏明*
片山　一朗*　吉岡　大輔**　戸田　宏一**　澤　芳樹**

Key words
アスピリン(Aspirin)，中毒性表皮壊死症(toxic epidermal necrolysis；TEN)，親子例，家族歴(family history)，ヒト白血球抗原(human leukocyte antigen；HLA)，免疫グロブリン大量療法(intravenous immunoglobulin；IVIG)

症例のポイント

- 自験例は拡張型心筋症の経過中，アスピリン内服後に中毒性表皮壊死症(toxic epidermal necrolysis，以下，TEN)を発症した．
- ステロイドパルス療法と単純血漿交換療法(plasma exchange，以下，PE)を併用し，さらに免疫グロブリン大量療法(intravenous immunoglobulin，以下，IVIG)を追加したことで，速やかに上皮化した．
- 自験例の父親にもアスピリンを含有する市販感冒薬を内服した後に，粘膜疹を発症した既往歴があった．
- 特定のヒト白血球抗原(human leukocyte antigen，以下，HLA)アリルを有する人において，特定の薬剤に対する重症薬疹を発症するリスクが高いことが知られるが，アスピリンについては不明である．
- 自験例とその父はアスピリンに対するDLST検査がともに陽性であり，父子で共通するHLAアリル(HLA-A*24：02，B*07：02)がTENの発症に関連した可能性が示唆された．

症例　49歳，男．
初診　2013年2月．

家族歴　父が22歳時にAPC(市販感冒薬，アスピリン/フェナセチン/カフェイン)を内服後に口腔，陰部の粘膜疹を発症した既往あり．

既往歴　45歳時にアジスロマイシンで薬疹(内服翌日に発症．詳細不明)．

現病歴　2013年1月初旬(第1病日)急性心不全のため他院に緊急入院し，第3病日から同院にてアスピリン100 mg内服が開始された．第7病日拡張型心筋症に伴う心不全が疑われ，当院心臓血管外科へ転院し，同日人工呼吸のため集中治療室(Intensive Care Unit，以下，ICU)へ入室した．第20病日に体外型左室補助人工心臓を装着したが，第23病日に出血に伴う血胸，縦隔血腫となり，再開胸止血術を施行し同時にアスピリンを中止した．止血が確認された第36病日にアスピリン内服を再開した．第37病日に上肢に紅斑が出現し当科へ紹介となった．第29病日より肺炎に対して投与されていたセフェピムによる薬疹を疑われ，同薬剤を中止し，ベタメタゾン酪酸エステルプロピオン酸エステルクリーム(アンテベートクリーム)を外用開始したが改善せず，第51病日に四肢に弛緩性水疱が出現したため，当科を再診した．

現症　体温38℃．両下腿を中心に両大腿，および両肘頭周囲に2 cmまでの暗赤色の紅斑が散在および融合(図1)し，中心には弛緩性水疱(図2)が

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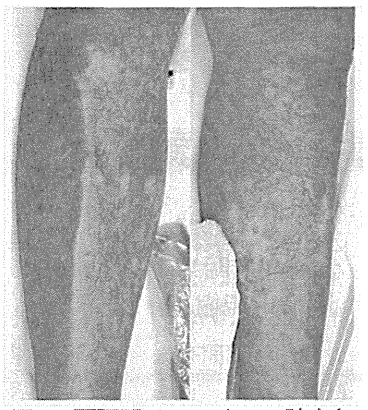

図1　両下腿 2 cmまでの暗赤色の紅斑が散在，融合していた．Nikolsky現象陽性で一部に水疱を伴っていた．

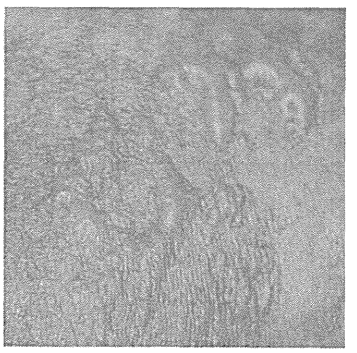

図2　左下腿の紅斑の中心に弛緩性水疱が存在していた．

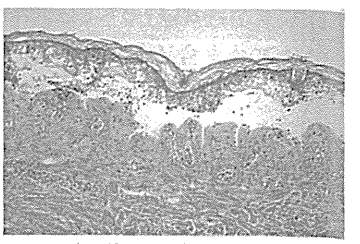

図3　病理組織学的所見．
表皮が全層性に壊死し，表皮下に水疱を形成し，表皮内から真皮上層にかけてリンパ球を中心とした炎症細胞が疎らに浸潤していた(H-E染色，×40，右大腿)．

みられた．両下腿にNikolsky現象陽性のatypical flat target lesion(図1)もみられた．紅斑および水疱の面積は体表面積の14％であった．顔面および体幹には皮疹がなく，粘膜疹は認めなかった．

臨床検査所見

白血球 21,510/μl(好中球 73.1％，リンパ球 6.2％，好酸球 13.9％)，AST 56 IU/l，ALT 95 IU/l，γ-GTP 80 IU/l，LDH 550 IU/l，BUN 30 mg/dl，Cre 0.87 mg/dl，CRP 4.42 mg/dl．好酸球増多および肝機能障害，炎症反応が認められた．

HSV-IgM 0.76，HSV-IgG 3.90，VZV-IgM 0.53，VZV-IgG 15.6，CMV-IgM 0.53倍，CMV-IgG 2.20倍，C7-HRP(−)，EB-VCA IgM＜10倍，EB-VCA IgG 80倍，EB抗EBNA 80倍，マイコプラズマ抗体(PA) 160倍．2週間後にペア血清を測定したが，これらの抗体価の上昇はなく，マイコプラズマ抗体は80倍と低下していた．

発症前に投与されていた8薬剤(アスピリン，セフェピム，レボフロキサシン，ミカファンギン，オメプラゾール，カルベジロール，バンコマイシン，メロペネム)について発症時に薬剤誘発性リンパ球刺激試験(drug-induced lymphocyte stimulation test，以下，DLST)を施行した．DLSTではアスピリンのみ234％と陽性であった．

病理組織学的所見

第51病日に右下肢の暗赤色の紅斑から皮膚生検を施行し，迅速病理診断を行った．表皮が全層性に壊死し，表皮下に水疱を形成していた(図3)．また，表皮内から真皮浅層にかけてリンパ球を中心とする炎症細胞が軽度浸潤していた．好中球や好酸球は少数認めるのみであった．

鑑別診断

ブドウ球菌性熱傷様皮膚症候群(Staphylococcal Scalded Skin Syndrome，以下，SSSS)：病理組織学的に表皮の全層性壊死を認めることから否定した．

診断確定

自験例では38℃の発熱，体表面積の14％にNikolsky現象陽性の紅斑ならびに水疱を認め，病理組織学的にSSSSを否定でき，本邦のTENの診断基準2005における主要所見3項目をすべて満たすことに加えて，迅速病理診断において表皮の全層性壊死を認めることから，第51病日再診時にTENと診断した．

治療と経過

第51病日再診時に上記のとおりTENと診断した．カルベジロールとワーファリンを除く投与されていた点滴薬，内服薬をすべて中止し，同日より3日間，ステロイドパルス療法(メチルプレドニゾロン1,000 mg/日 3日間)とPE療法を併用した．

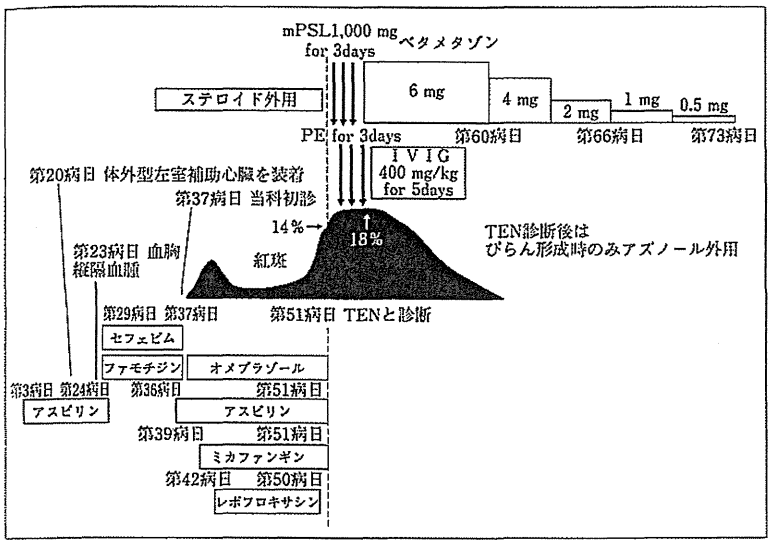

図4 臨床経過
ステロイドパルス療法，PE療法を施行も病勢は進行していたが，IVIG療法開始後，速やかな上皮化を認めた．

また，外用薬塗布時の物理的刺激による水疱，びらん・潰瘍の拡大を避けるため，紅斑には継続的な外用療法を施行せず，びらん形成時のみジメチルイソプロピルアズレン軟膏(アズノール軟膏)を外用した．解熱はしたものの，紅斑・水疱は徐々に18%まで拡大した．そこで第54病日より5日間のIVIG療法(400 mg/kg/日) を開始したところ，速やかな上皮化がみられた．ステロイドパルス療法の後療法として，ベタメタゾン6 mgを第54病日より開始し，その後3週間程度で漸減中止した(図5)が，再燃を認めていない．

原因薬剤を検索する目的で，発症2カ月後にDLSTを行った8薬剤について，パッチテストならびにスクラッチパッチテストをそれぞれ施行した．パッチテストはすべて陰性であったが，スクラッチパッチテストではセフェピム(10%および1%)においてICDRG基準で＋であった．

考 按

自験例はアスピリンが原因薬と考えられたTENの1症例であった．今回われわれは，原因薬を検索する目的で，TENの診断前に投与されていた8薬剤についてDLST，パッチテスト，スクラッチパッチテストを施行した．DLSTではアスピリンのみ，スクラッチパッチテストではセフェピムのみがそれぞれ陽性であった．しかし，セフェピムについてはTEN発症の2週間前から中止しており，否定はできないものの原因薬剤であった可能性は低いと考えた．レボフロキサシン，ミカファンギン，オメプラゾールの3剤については紅斑発症後に投与開始されていた．また，有意なウイルス抗体価の上昇ならびに再活性化を認めなかった．以上からアスピリンがTENの原因薬であると考えた．

治療としては，まずステロイドパルス療法とPE療法を併せて行ったが，紅斑および水疱は拡大した．そこで第54病日よりベタメタゾン6 mg/日にIVIG療法を追加して行ったところ，紅斑および水疱の拡大は止まり，速やかな上皮化を認めた(図5)．IVIG療法はStevens-Johnson症候群(Stevens-Johnson syndrome，以下，SJS)やTENに保険適応がなく，その効果については国内外で評価が分かれるが，開始時期としてなるべく早期が望ましく，ステロイド大量療法に反応しない場合は3～4日以内に始めるべきであり，投与量については最低400 mg/kg以上の投与量が必要であるとされている[1]．また，一般的には2日程度はステロイドパルス療法やPE療法の治療効果を確認した後にIVIG療法を開始するか否か判断するが，自験例では重篤な心疾患を合併していたことから効果判定を待てないと判断し，翌日よりIVIG療法を開始した．そのため，自験例におけるIVIG療法の効果を前療法と区別して評価することは難しいが，結果として良好な経過が得られた．

アスピリンによる薬疹の本邦における報告例は福田の薬疹情報第14版[2]によると，自験例を含めて29例であった．そのうち，即時型反応と考えら