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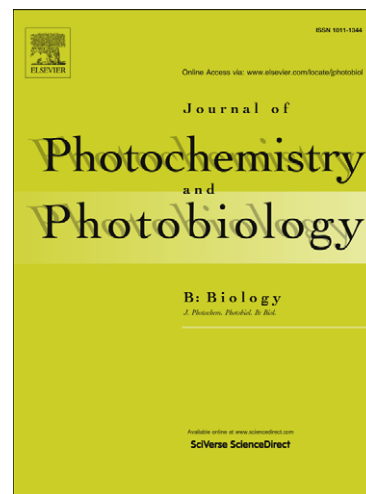
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Title:

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

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Key words

Ketoprofen, butyl methoxy dibenzoylmethane, UVA, photoallergy, photocontact dermatitis, plaster

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 prohaptent 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 prohaptent theory suggests that UV simply converts photosensitizing substances into ordinary haptent, 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 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 (DHBB) was purchased from BASF Japan Ltd (Tokyo, Japan). Hydrophobic particle (mean size: 20 x 100 nm) of titanium dioxide (TiO₂) 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 μ m, 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/cm² 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 μ L of 2% KP alone or 2% KP plus various concentrations of BMDBM, OX, DHBB or TiO₂ in acetone/olive oil (4:1), and subsequently irradiated with 20 J/cm² 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 μ L of phosphate buffered saline (PBS) containing 20 μ Ci [methyl-³H] thymidine (³H-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 1,100 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. ^3H -TdR incorporation was measured by β -scintillation counter (Hitachi Aloka Medical, Ltd., Tokyo, Japan). Percentage of control was calculated according to the following formula: % of control = (sample - vehicle control) / (KP alone control - vehicle control) x 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 cm². 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 3,000 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 tetrahydrofuran, 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/cm² 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 (1cm²) 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: edema rate (%) = [(paw volume after adjuvant injection) / (paw volume before adjuvant injection) - 1] x 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 TiO₂ 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 ³H-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.

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/cm², 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/cm² 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 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 TiO₂, is more photostable and less likely to have its photoallergy than UVA absorbers. Although the inhibitory ability of TiO₂ was weaker than BMDBM, we also manufactured a KP patch formulation containing TiO₂. However, the inhibitory effect of TiO₂ addition was not observed in the animal model (data not shown). Since TiO₂ in organic solvent reduced the KP photosensitivity, TiO₂ 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.

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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Figure legends

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

Figure 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).

Figure 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).

Figure 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.

Figure 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.

Figure 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.

Table legend

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

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

Figures

UVA absorber, butyl methoxy dibenzoylmethane included in topical ketoprofen formulation reduces the photoallergic reactions

Fig. 1

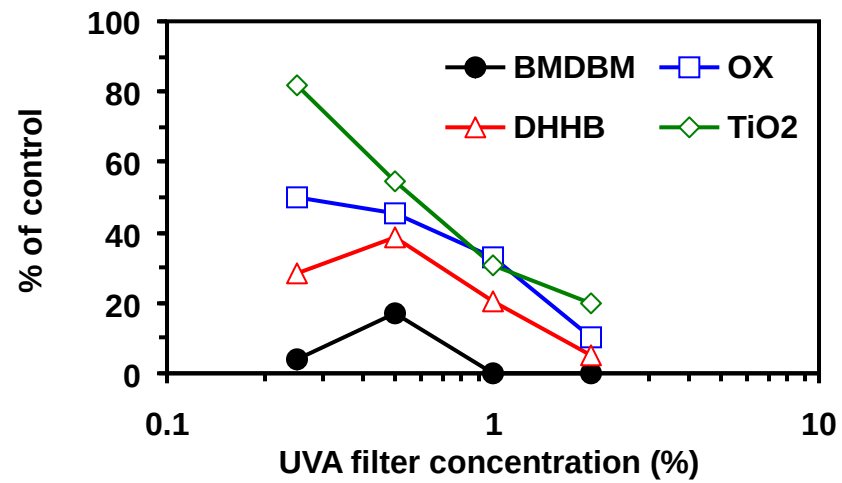
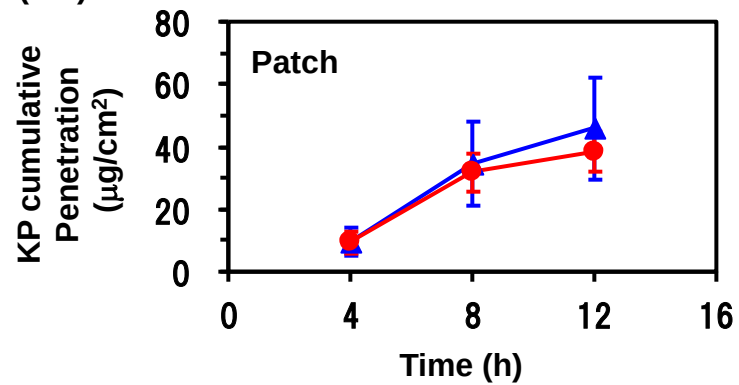
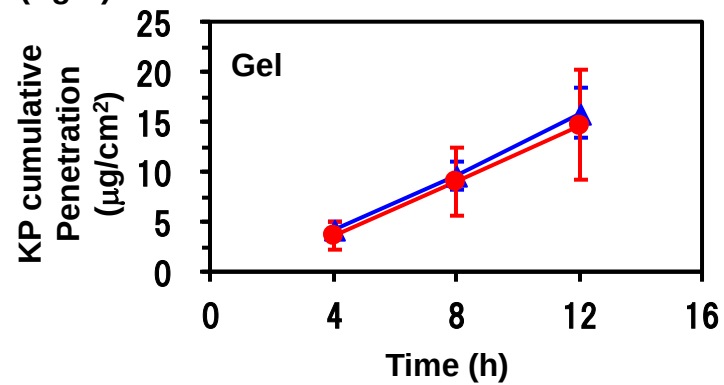


Fig. 2

A (left). Mouse Skin.



A (right). Mouse Skin.



B. Human Skin.

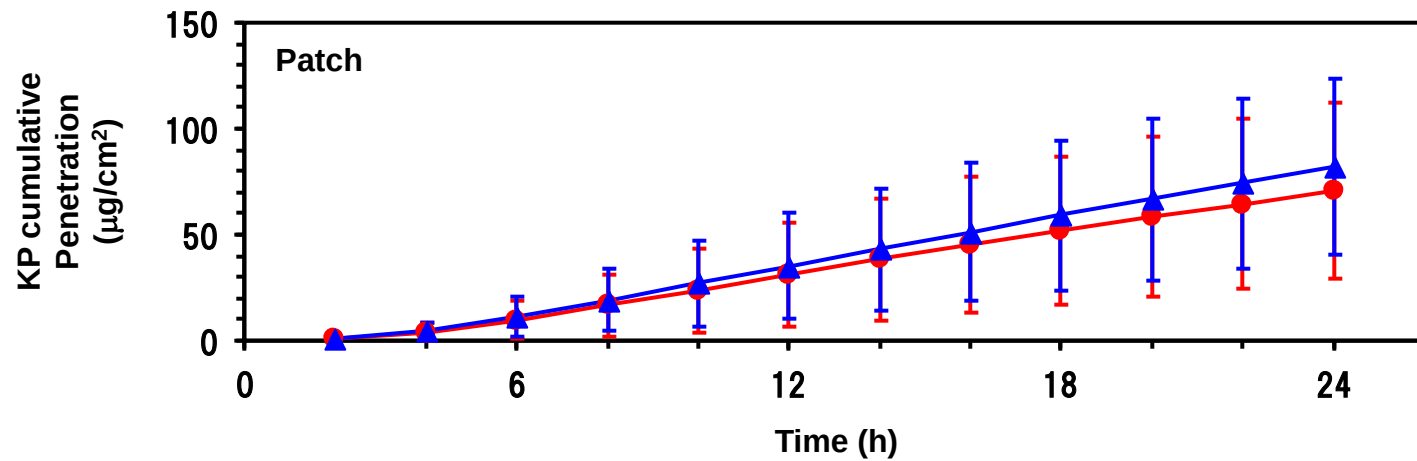


Fig. 3

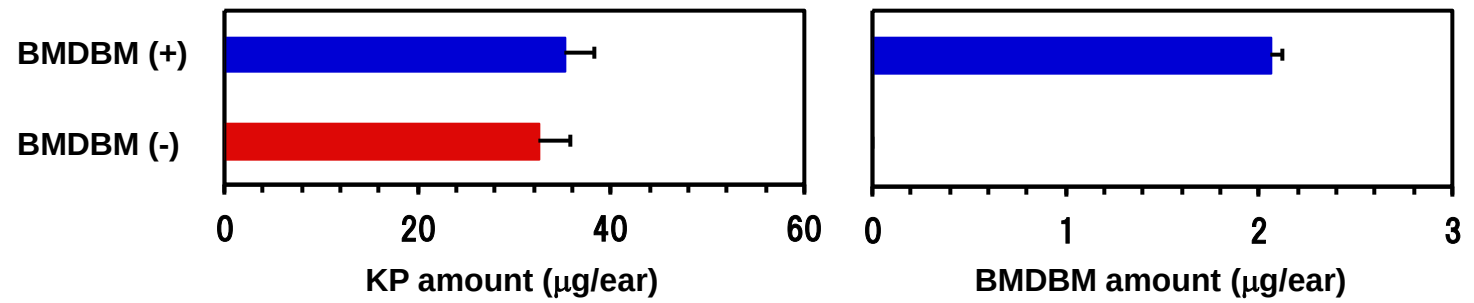


Fig. 4

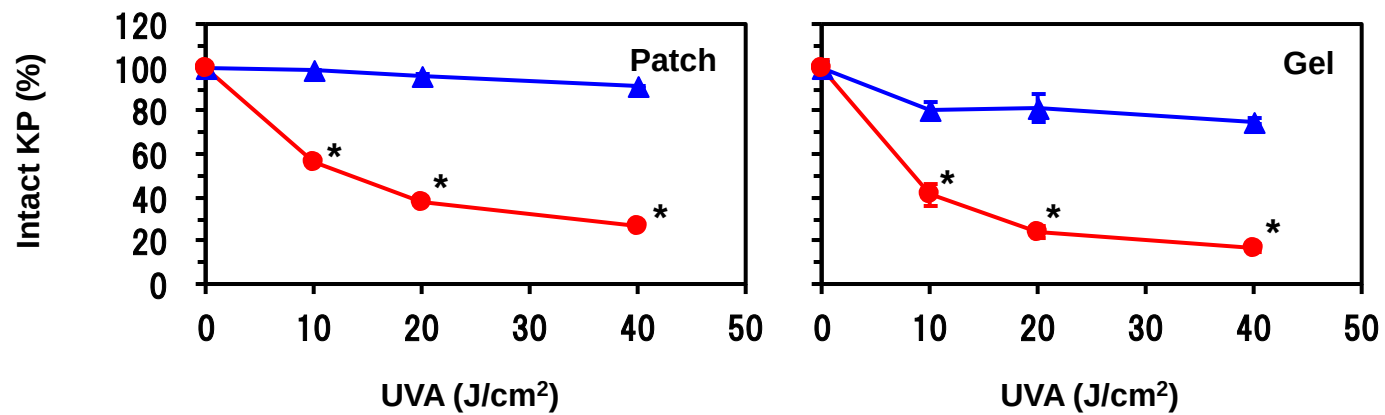


Fig. 5

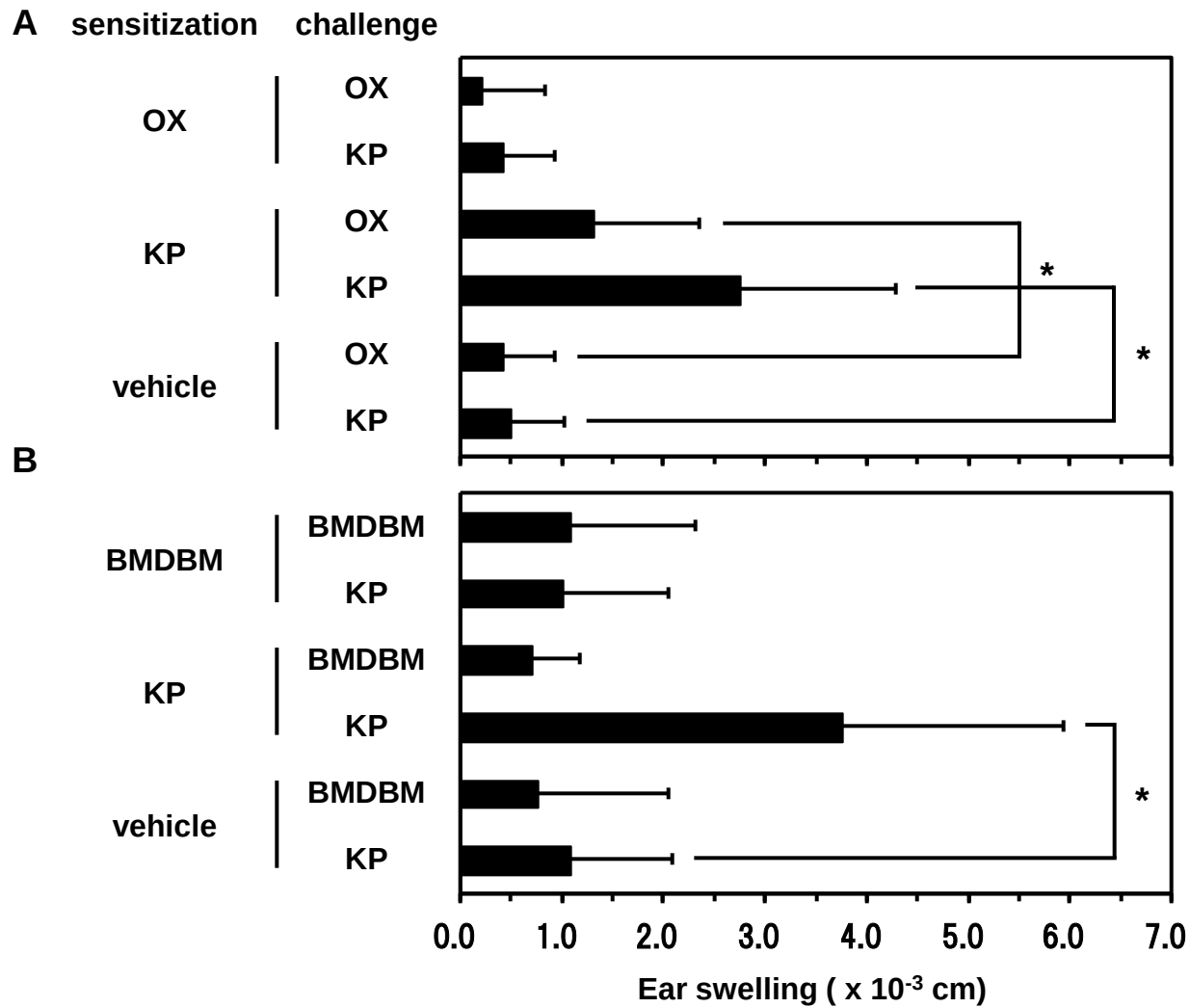
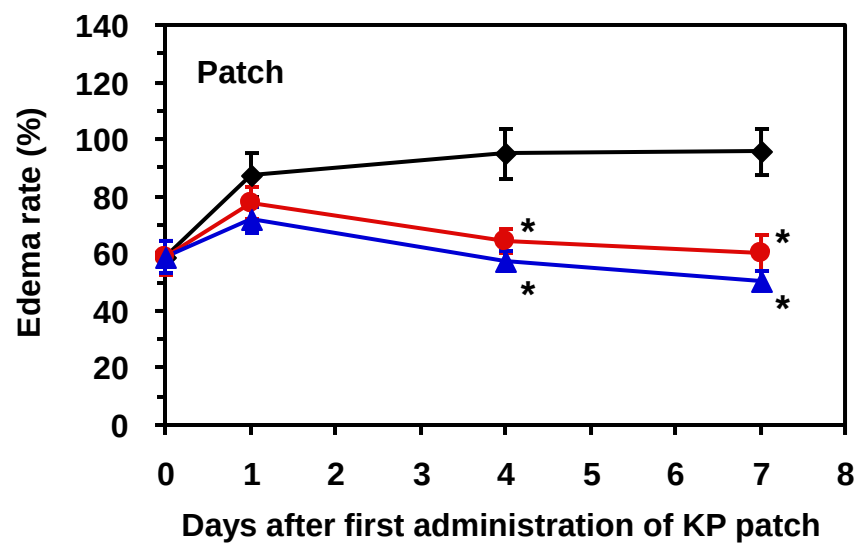


Fig. 6



Table

UVA absorber, butyl methoxy dibenzoylmethane included in topical ketoprofen formulation reduces the photoallergic reactions

Table.1

Group	photosensitization	photochallenge	N	mean score	
				24h	48h
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

Highlights

- We examine the inhibitory effect of UVA filters for ketoprofen photocontact dermatitis.
- Butyl methoxy dibenzoylmethane is the most effective UVA filter.
- Patch and gel containing the UVA filter retain the pharmacokinetic performance.
- The UVA filter in both topical formulations suppresses ketoprofen photosensitivity.
- The UVA filter is not photo-cross-reactive with ketoprofen.