

Research Article

Topical Lyogel Containing Corticosteroid Decreases IgE Expression and Enhances the Therapeutic Efficacy Against Atopic Eczema

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Abstract. Hydrocortisone cream intended for atopic eczema often produces unwanted side effects after long-term use. These side effects are essentially due to repeated percutaneous administration of the medication for skin dermatitis, as atopic eczema is a relapsing disorder. Hence, there is a need to develop a new hydrocortisone formulation that will deliver the drug more effectively and require a reduced dosing frequency; therefore, the side effects could be minimized. In this study, a hydroxypropyl methylcellulose (HPMC) lyogel system based on 80% organic and 20% aqueous solvents containing 1% hydrocortisone was formulated. The hydrocortisone lyogel physicochemical characteristics, rheological properties, stability profile, and *in vitro* Franz cell drug release properties, as well as the *in vivo* therapeutic efficacies and dermal irritancy in Balb/c mice were investigated. The HPMC lyogel appeared clear and soft and was easy to rub on the skin. The lyogel also showed a higher drug release profile compared with commercial hydrocortisone cream. Similar to the cream, HPMC lyogels exhibited pseudoplastic behavior. From the mouse model, the hydrocortisone lyogel showed higher inflammatory suppressive effects than the cream. However, it did not reduce the transepidermal water loss as effectively as the control did. The dermal irritancy testing revealed that the hydrocortisone lyogel caused minimal irritation. In conclusion, HPMC lyogel is a promising vehicle to deliver hydrocortisone topically, as it showed a higher drug release *in vitro* as well as enhanced therapeutic efficacy in resolving eczematous inflammatory reaction compared with commercial cream.

KEY WORDS: atopic eczema; eczematous inflammatory reaction; hydrocortisone; IgE; lyogel; transepidermal water loss.

INTRODUCTION

The World Allergy Organization (WAO) states that eczema affects 2–5% of children and approximately 10% of young adults worldwide, and atopic eczema is one of the most common dermatoses (1). Atopic eczema is an inflammatory, chronically relapsing, noncontagious, and extremely pruritic skin disease that has a demonstrable IgE association. The eczema lesions appear in various forms from the collections of fluid in the skin (vesicles) to the gross thickening of the skin (lichenification) on a red skin background (erythema). Other clinical presentations of eczema include crusting, scaling, cracking, and swelling of the skin. Eczema is not life-threatening, but the constant scratching due to the intense itching may result in skin damage, secondary infections, and sleep disturbances as well as frequent visits to physicians, which contributes to the burden of disease to the patients (2,3). Studies have also shown a marked increase in serum IgE levels in atopic eczema patients, which is the most essen-

tial biochemical parameter in patients suffering from atopic eczema (4).

Topical corticosteroids are the mainstay of treatment for eczema because of their anti-inflammatory action and overall safety when used appropriately. According to Raimer, group VII corticosteroids, which are the least potent and include hydrocortisone, are generally used to treat atopic eczema (5). However, long-term application of hydrocortisone results in significant side effects (6). The common local side effects include striae, pigment changes, and skin atrophy (6). Prolong steroid use may exert systemic adverse effects, such as hypothalamic–pituitary–adrenal axis suppression, Cushing’s syndrome, femoral head osteonecrosis, and cataracts (7). Hence, a new vehicle for corticosteroids is being sought to decrease the frequency of application and thus minimize side effects.

The gelling system is a medium for drug dissolution. Its three-dimensional swollen network and thermodynamic nature have essential functions in their diffusional behavior, molecular mesh size changes, and the molecular stability of active ingredients (8,9). Hydrogel is often preferred by patients because of its non-oily texture, cooling sensation, and ease of removal through washing. However, hydrogels have a limited ability to improve barrier properties and are unable to solubilize hydrophobic drugs such as hydrocortisone (10). This insolubility problem is often addressed by adding 10–30% of

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an organic solvent such as propylene glycol into the hydrogel formulations. Lyogel ("lyo" means solvent in Greek), developed in our laboratory, is a new gel system in which the pores are filled with both aqueous as well as nonaqueous solvents. Lyogel is not the same as organogel as the latter consists of only organic solvent. And in lyogels, the fraction of organic solvents is higher than that of aqueous solvent. Lyogel has several advantages over hydrogel. The former is expected to have minimal risk for microbial growth, as it comprises a very low amount (only 10–20% *v/v*) of aqueous solvents compared to hydrogel. Lyogel can also enhance the penetration of drug into the skin due to the penetration-enhancing properties of the organic solvent, such as propylene glycol (11,12).

In this study, a lyogel formulation containing 1% corticosteroid for the treatment of atopic eczema was developed and characterized. HPMC (4–8% *w/v*) was used as the gelling agent because it can form a stable swollen gel network in propylene glycol, while hydrocortisone (1% *w/v*) was used as the model drug. The physicochemical characteristics, storage stability, and rheological properties of the lyogels were investigated. The *in vitro* Franz cell diffusion of hydrocortisone lyogels through excised mouse skin was determined. Then, the *in vivo* therapeutic efficacies of the lyogel in eczema-induced Balb/c mice and dermal irritancy were investigated. One percent commercial hydrocortisone cream was used as the positive control.

MATERIALS AND METHODS

Materials

Hydrocortisone, hydroxypropyl methylcellulose (HPMC, methoxy group ~28–30%, analytical grade) was obtained from Sigma-Aldrich (USA). Propylene glycol 4000, disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from R & M Chemicals (UK). Dinitrofluorobenzene (DNFB), acetone/olive oil, diethylether, hematoxylin, eosin, and xylene were from Merck (Germany). An ELISA kit was obtained from USCN Life Sciences Inc. (USA). Hydrocortisone cream was from CCM Pharmaceutical (Malaysia).

Methods

Lyogel Formulation

The three lyogel formulations are listed in Table I. The HPMC powder was added to the solvents under continuous stirring at 60°C until the powder was dissolved. The gel was

Table I. Formulation of 1% *w/w* Hydrocortisone HPMC Lyogels (10 g)

Composition	HPMC 4% <i>w/w</i>	HPMC 6% <i>w/w</i>	HPMC 8% <i>w/w</i>
HPMC (g)	0.4	0.6	0.8
Propylene glycol (mL)	8	8	8
Water (mL)	2	2	2
Hydrocortisone (g)	0.1	0.1	0.1

HPMC hydroxypropyl methylcellulose

removed from the heat, and hydrocortisone was added. The mixture was left to cool to room temperature (25°C).

Rheological Measurement

The rheological properties of the hydrocortisone lyogels were determined using a cone-and-plate rheometer, geometry of 20 mm/2° (Bohlin Gemini, UK), at a constant shear rate of 500 s⁻¹. The shear rate was increased from zero to 100 s⁻¹ in 3 min followed by a constant rate decrease to zero in the same time interval. Typically, three independent measurements were collected for each sample. The average apparent viscosity at the flow curve apex (i.e., at 100 s⁻¹) of each sample was determined. The same procedures were also performed on hydrocortisone cream. The apparent viscosity of lyogel and the cream was compared using an unpaired *t* test. *p* values less than 0.05 were considered significant.

Stability Test

The stability test was performed by placing the lyogels in three different temperature environments: 4, 25, and 40°C. The lyogels were assessed at intervals of 1, 2, 3, 4, and 8 weeks. The observed criteria included the appearance, texture, homogeneity, odor, and color.

In Vitro Permeation

The *in vitro* permeation study was performed using Franz diffusion cell (PermeGear Inc., USA) using a 0.45- μ m cellulose acetate membrane and excised Balb/c mouse skin.

Preparation of Excised Mouse Skin. All animal studies were approved by the Animal Ethics Committee Universiti Kebangsaan Malaysia (UKMAEC) with the approval number FF/2013/FERN/15-MAY/520-AUG.-2013-JAN.-2014. Thirty Balb/c mice aged 5 weeks (19–22 g) were obtained from Animal House, UKM Bangi. Twenty-four mice were allocated for *in vivo* investigation, while six mice were used for dermal irritancy testing. The mice were assigned randomly into four groups with six mice per group. The six mice in each group were subdivided equally into two cages (*n*=3). For the dermal irritancy group, the six mice were caged individually. The mice were allowed to adapt to the new environment for 1 week with unrestricted food and water access with a 12-h light/dark cycle. Saw dust was used as bedding for the first 2 weeks and corncob for the third week.

Six Balb/c mice were sacrificed through a drug-induced method using diethylether. The hair on the dorsal skin was shaved using an electric razor. The skin was then surgically removed and cut into smaller pieces with a 1.5-cm diameter before being soaked in a PBS solution for 15–30 min prior to the *in vitro* permeation study.

In Vitro Skin Permeation Experiment. This experiment was performed using 9-mm Franz diffusion cells, which comprised two compartments: the upper donor or flat ground oring and the lower receptor chamber with a 5-mL volume. A sampling arm protruded from the receptor chamber, which provided the site for sample collection. The 0.45- μ m pore size cellulose acetate membrane with a 1.3-cm diameter was sandwiched between the upper donor and the lower receptor

chamber. The chamber contained a magnetic stir bar to provide constant stirring.

The Franz cell was maintained at 37°C for 15 min prior to adding PBS to the receptor chamber. A PBS solution was freshly prepared in a 1000-mL Schott bottle and immersed in a water bath to maintain the temperature at 37°C. The receptor chamber was then filled with PBS until the calibration mark on the sampling arm. The membrane was mounted carefully on top of the receptor chamber using forceps, and a flat ground o-ring was placed over each membrane and clamped tightly with a stainless steel clamp. Any bubbles that formed beneath the membrane were observed carefully and removed. The receptor medium was equilibrated to 37°C by a circulating water jacket throughout the experiment. The prepared Franz cell that was filled with PBS was equilibrated for 30 min prior to sample loading into the donor chamber.

After 30 min of equilibration, 0.5 g of the lyogel was loaded on the membrane through the upper donor chamber. Parafilm was used to occlude the donor chamber and the sampling arm to prevent the evaporation of receptor medium from the sampling port after loading the sample. A volume of 0.2 mL of receptor medium was drawn using a syringe at predetermined time intervals, which were 60, 120, 180, 240, 300, 360, 420, and 480 min. A similar volume of PBS solution was replaced to maintain the sink condition.

The sample was assessed using a UV spectrophotometer (Shimadzu 1800, Japan) at a wavelength of 242 nm to obtain the absorbance value at each time interval to determine the amount of hydrocortisone that permeated through the membrane. The cumulative amount of hydrocortisone that permeated through the membrane was plotted as a function of time (minutes). The rate of cumulative amount of drug release (which was derived from the gradient of steady state drug release) from lyogel and cream across the cellulose acetate membrane and mouse skin was compared using an unpaired *t* test. *p* values less than 0.05 were considered significant.

In Vivo Investigations

After a 1-week adaptation, the dorsal skin of the mice was shaved using an electric razor to remove the hair. A total of 24 Balb/c mice were divided into four groups with six mice per group (*n*=6). The mice in group I were treated with HPMC hydrocortisone lyogel, while group II was treated with drug-free lyogel. Group III acted as the positive control group and was administered commercial hydrocortisone cream, while group IV was administered saline solution and acted as the negative control group. Twenty-four hours prior to induction, the shaved skin was evaluated for erythema and transepidermal water loss (TEWL) using Mexameter® and Tewameter® probes (TM 310 Courage & Khazaka, Germany), respectively.

Induction of Eczema and Treatment. The induction of eczematous skin lesion was performed on all groups through the application of 100 µL of 0.5% DNFB solution in 4:1 acetone/olive oil. The induction was applied daily for 7 days in a rotated manner on the dorsal skin to obtain a uniform skin

lesion of eczema. The mouse was returned to the cage, and cage-side observations of the skin lesions of eczema were performed daily for 1 week. The observed parameters included erythema, edema, and scaling, which were scored as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The skin condition of the mice was imaged daily to evaluate the development of eczema by two experienced evaluators in a blinded manner using the same scoring system. After the 7th day of induction, the dorsal skin of the mice was again assessed for erythema and TEWL.

The treatment of the experimental group was initiated after the 7th day post-induction of eczema. Group 1 mice were applied with hydrocortisone lyogel using the fingertip unit (FTU) method, as thinly as possible, based on total shaved area, which varied. The second and third groups were also applied using a similar principle, whereas the fourth group of mice was applied with 1–2 drops of saline solution using a plastic pipette. After application, the mice were returned to the cage, and cage-side observations of skin eczema lesions were performed daily for 7 days; the lesions were scored using the aforementioned scoring system. After the 7th day of treatment, the dorsal skin of the mice was assessed for erythema and TEWL using the mexameter and texameter probes, respectively.

After the 7th day of treatment, the mice were sedated with diethylether prior to the collection of blood and skin excision. Blood was withdrawn from the eyes using the retro orbital technique. Then, the skin was excised using scissors. The fatty layer beneath the skin was carefully removed. Soon after the skin was excised, the mice were sacrificed through cervical dislocation. While drawing the blood and excising the skin, the mice should be alive to ensure functional physiological systems and avoid inaccuracies when measuring the parameters of interests. The dead mice were placed into biohazard plastic bags in the freezer.

Enzyme-Linked Immunosorbent Assay (ELISA). One milliliter of blood was drawn from the eyes of mice retro-orbitally using a hematocrit capillary tube and collected in centrifuge tubes. Serum was obtained by centrifugation (Hettich Zentrifuge, Germany) at 4000 rpm for 15 min at 4°C. The serum was collected and transferred into Eppendorf tubes and stored at 20°C until use. Total IgE levels in serum were measured through an enzyme immunoassay. The concentration of total IgE in the sera was measured using a 96-well sandwich enzyme-linked immunosorbent assay (ELISA) with mouse serum (IgE). The IgE concentration for each group was analyzed using ANOVA, and *p* values less than 0.05 were considered significant.

Histopathology Analysis. The skin was raised and excised using scissors. The fatty layer beneath the skin was carefully removed. For histological evaluation, the excised skin tissues were fixed in 10% formalin solution for 2 days at room temperature. Specimens were placed into cassettes and dehydrated in increasing concentrations of ethanol, toluene, and paraffin to process the skin tissues. The tissues were then embedded in paraffin using paraffin-embedding center (Leica EG1160, Germany) at 60°C for the blocking process. Two blocks were prepared for each tissue. The blocks were allowed to freeze on a cold plate for 1 h. Then, they were left at room temperature for 24 h prior to sectioning with a microtome.

Sections of 5 μm were obtained using a microtome (Leica RM2135, Germany) for one block, and three slides for each tissue were prepared by the fishing method and left on the fisher slide warmer, which was maintained at 41°C overnight. The slides were arranged in the slide rack and underwent the staining process using hematoxylin and eosin and were later immersed in xylene until the tissues had a clear appearance. The specimens on the slides were mounted using DPX mountant before being analyzed under a microscope. The tissues were examined under a Leica CTR MIC (Leica, Germany) bright-field microscope, and images were captured using a Pixelink (Leica, Germany) digital camera attachment. The histology images were analyzed by a histologist from the anatomy department, UKM Kuala Lumpur.

Dermal Irritancy Testing

Balb/c mice ($n=6$) were subjected to dermal irritancy testing and were individually caged. On day 0, the left ear of each mouse was applied 10 mg of 0.1% hydrocortisone lyogel, while the right ear was left untreated as a control for 6 days. The next day (day 1), the erythema and ear swelling tests, which represents edema, were evaluated using a magnifying glass and a digital caliper (Mitutoyo, Japan), respectively. The development of erythema was scored using the Uttley test: 0—no erythema development; 2—barely visible with few blood vessels and no erythema development; 4—main blood vessels were visible and slight erythema development over a third or the base of the ear; 6—main blood vessels were more obvious and slight erythema development (13). Irritation potential was calculated using Eq. (1) as follows:

$$\text{Resultant indices} = \frac{A \times B}{\text{number of observation days}} \quad (1)$$

where A and B represent the erythema value and the corresponding day, respectively.

The ear swelling test was performed by measuring the ear thickness using a digital caliper. The erythema and ear swelling tests were performed for 6 days during which the scoring was performed daily. The data are presented as the average ear swelling for six mice.

RESULTS AND DISCUSSION

Gel Characterization and Stability Test

Figure 1 shows a representative image of the HPMC lyogels produced in this study. The HPMC lyogels were colorless, clear, and transparent as well as odorless. The HPMC 4 and 6% lyogels were able to flow when the container was tilted at 45°; however, HPMC 8% lyogel remained stiff. Sampling of all lyogels was easy, and, upon application to the skin, the gels were soft and easy to spread. However, HPMC 8% was slightly sticky when applied to the skin.

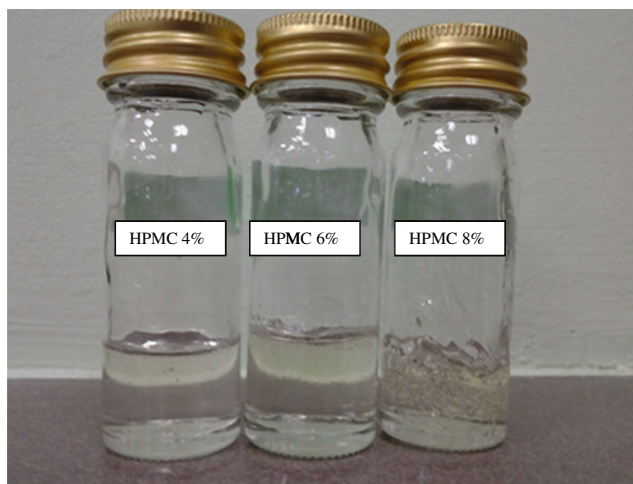


Fig. 1. Hydroxypropyl methylcellulose (HPMC) lyogels stored at room temperature

In the gel development, the concentration of gelling agent determines the consistency of the gel depending on its intended purposes. According to Ofner III and Klech-Gelotte (14), HPMC concentrations between 2 and 10% w/v are used to prepare hydrogels. For lyogel development, HPMC concentrations of 4, 6, and 8% w/v were ample to form a stable formulation for topical use. PG and water were chosen to be solvated in HPMC (with higher ratio of PG) as HPMC was able to be dissolved in these solvents. PG is frequently used in topical products as it has the capacity to solubilize hydrophobic drugs as well as improve skin penetration behavior (11).

When stored at 4°C, the HPMC lyogels were stiff and did not flow, while at 25 and 40°C, all HPMC lyogels were able to flow. After week 8 of storage, all lyogels remained unchanged and retained the appearance, texture, and flowability from their initial state. These data indicated that the HPMC lyogels were physically stable, which is a desirable characteristic of topical gel formulations.

Rheology

Rheology is the study of the flow and deformation of materials under an application of force. The selection of concentration of gelling agents used in this study was based on the satisfactory consistency of gel for topical application observed during the pre-formulation studies. Figure 2 shows the flow curve of lyogels containing hydrocortisone and a commercial hydrocortisone cream. From the graph, all HPMC lyogels showed pseudoplastic flow, i.e., the viscosities gradually decreased with an increased shear rate (15). For topical application, a gel should exhibit shear-thinning properties, i.e., be thick throughout the shelf life and become thin when rubbed on the skin. The shear-thinning properties indicated that lyogels did not flow easily at low shear stress, but thinned as shear was applied and is thus suitable for topical application. As expected, the apparent viscosities increased as the concentration of gelling agent increased from 4% (0.16 Pa.s), to 6% (0.45 Pa.s), and to 8% HPMC (1.55 Pa.s). The cream also exhibited pseudoplastic flow and had an apparent viscosity of 1.16 Pa.s.

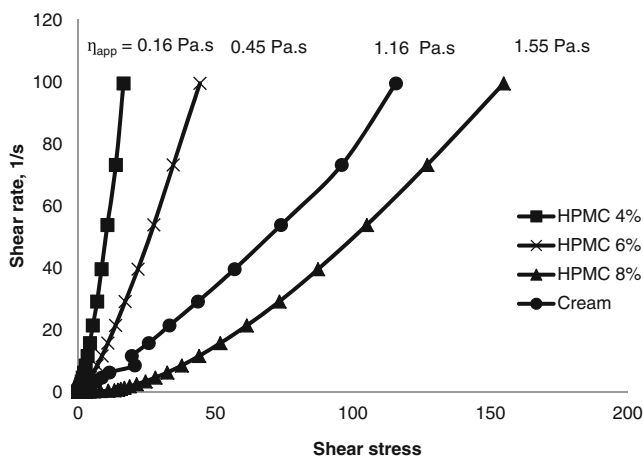


Fig. 2. Flow curves of HPMC hydrocortisone lyogels and commercial hydrocortisone cream

In Vitro Drug Release Test

Figure 3 shows the cumulative amount of hydrocortisone that permeated through mouse skin from the lyogels and commercial hydrocortisone cream. The lyogel formulations had a greater permeation of hydrocortisone compared with the commercial cream.

The drug flux was measured from the linear section of the cumulative amount of hydrocortisone release curve, 60–300 min to provide a value for flux ($\text{mg}/\text{cm}^2/\text{h}$). The drug fluxes for hydrocortisone lyogels were 0.0047, 0.0038, and 0.0030 $\text{mg}/\text{cm}^2/\text{h}$ for 4, 6, and 8% HPMC lyogels, respectively, while the drug flux of the commercial cream was 0.0024 $\text{mg}/\text{cm}^2/\text{h}$. The drug fluxes of lyogels were significantly different from the cream ($p < 0.05$).

The hydrocortisone appeared to be more readily release from the lyogels compared with the cream. The flux values corresponded with the lyogel viscosity, i.e., the average drug flux decreased as the gel viscosity increased. The highest drug flux formulation, 4% HPMC lyogel, was selected for the *in vivo* studies.

In Vivo Investigation

Transepidermal Water Loss, Erythema, and Skin Lesions

In vivo investigations were performed to evaluate the efficacy of the lyogel in treating eczema. Efficacy was assessed in terms of membrane integrity, infiltration of inflammatory mediators, serum IgE levels, as well as symptomatic improvement. Transepidermal water loss (TEWL) is a parameter for determining the moisture barrier function of skin as well as assessing the nature of skin suffering from eczema because it arises when the skin barrier is disrupted (16). Apart from TEWL, erythema (skin redness) is also an essential clinical parameter in eczematous skin lesions (2).

Before eczema induction, the TEWL for all groups was measured at 3.3–6.0 g/Hm^2 . After induction, the TEWL increased markedly approximately 10-fold to 41–52 g/Hm^2 , indicating that the skin barrier function was disrupted due to DNFB. The skin was also observed to be dry, wrinkled, and excoriated due to scratching by the mice. After treatment, all

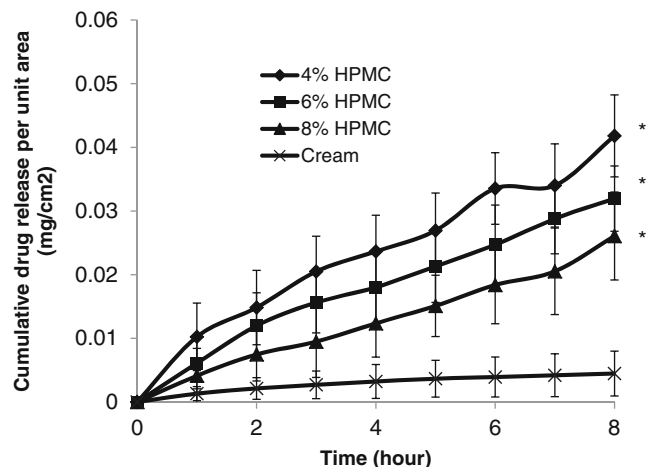


Fig. 3. Cumulative amount of hydrocortisone released against time permeated through mice skin. *Indicates significant difference compared to hydrocortisone cream ($p < 0.05$)

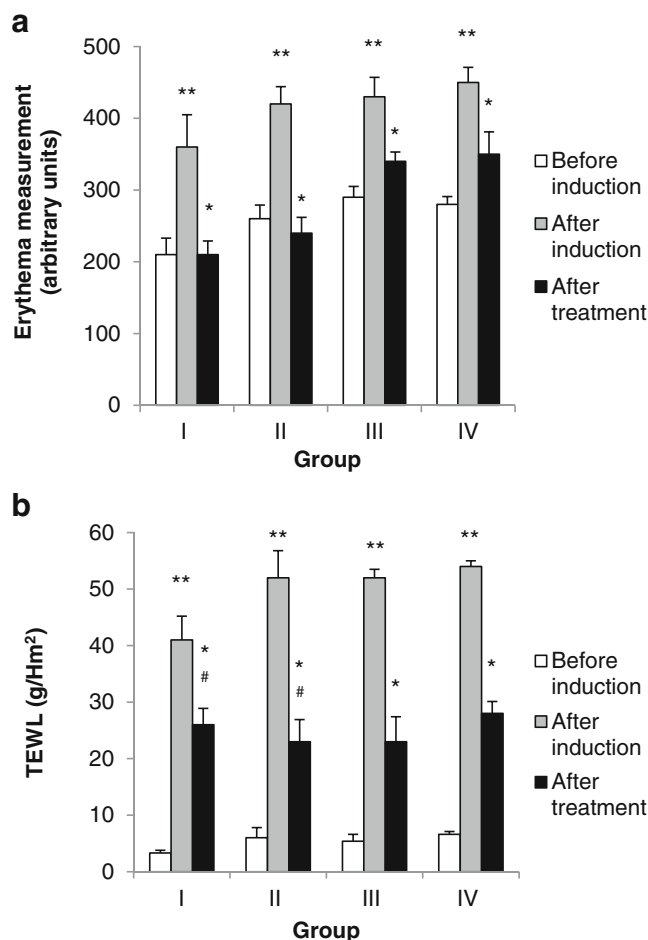


Fig. 4. **a** Transepidermal water loss (TEWL) and **b** erythema measurements of mice skin before eczema induction, after induction, and after treatment ($n=6$). *Indicates significant difference ($p < 0.05$) compared to after induction. **Indicates significant difference ($p < 0.05$) compared to before induction. #Indicates significant difference ($p < 0.05$) compared to groups III and IV

groups showed a significant decrease in TEWL (Fig. 4a); there was no significant difference between the groups. For erythema measurement, all groups developed redness upon DNFB application, which is due to capillary dilation as a result of the inflammatory reaction induced by DNFB. The erythema measurement by the Tewameter showed a significant reduction in redness after treatment (Fig. 4b). The lyogels (groups I and II) showed significantly lower erythema values compared with the controls (groups III and IV).

The results of TEWL and erythema evaluation suggested that the hydrocortisone lyogel did not effectively improve the skin barrier but did treat the inflammatory manifestation of the skin. The remaining groups, i.e., treatments with lyogel-free drug, commercial cream, and saline water, improved the skin barrier. However, they were not as effective as hydrocortisone lyogel in reducing redness.

The skin lesion evaluation was performed by two experienced evaluators to avoid bias. Before treatment, the scoring by the evaluators yielded similar results and showed no significant difference between the groups. When the skin lesion scoring was compared before and after treatment for each group, the skin lesions of groups II, III, and IV mice were significantly worse compared with group I (Fig. 5a). This result suggested that the treatments were not effective in reducing eczematous lesions symptomatically in contrast to the group I mice. The hydrocortisone lyogels suppressed the inflammation cascade activated by DNFB. The intervariability in the statistical analysis of the two evaluators for the skin lesions of group 1 mice showed a different perception of the skin lesions between the individuals. The difference in the scoring of skin lesions after a 1-week treatment between the two evaluators suggested that knowledge and experience in a specific field are of crucial importance for an accurate analysis, and differences in knowledge level and experience yield different perceptions and judgments.

Serum IgE Levels

The results of the serum IgE level measurements are shown in Fig. 5b. The serum IgE levels of group I mice were lower than those of the mice in groups II and III but showed no significant difference from group IV mice after a 1-week treatment period. This result suggests that the hydrocortisone lyogel was more effective in resolving the inflammatory reaction associated with eczema compared with commercial cream. The lower IgE levels of the negative control group (group IV) were possibly due to inadequate serum proportion due to hemolysis of red blood cells during blood collection. The pretreatment serum IgE levels were not measured to avoid killing the mice unintentionally during the invasive blood collection procedure.

Histopathology Analysis

Figure 6 shows the microscopic structure of skin tissues for groups I, II, III, and IV mice under $\times 2.5$ magnification. The membrane of eczematous skin (shown by the dashed arrow) of group I mice was intact and had a thickness similar to the

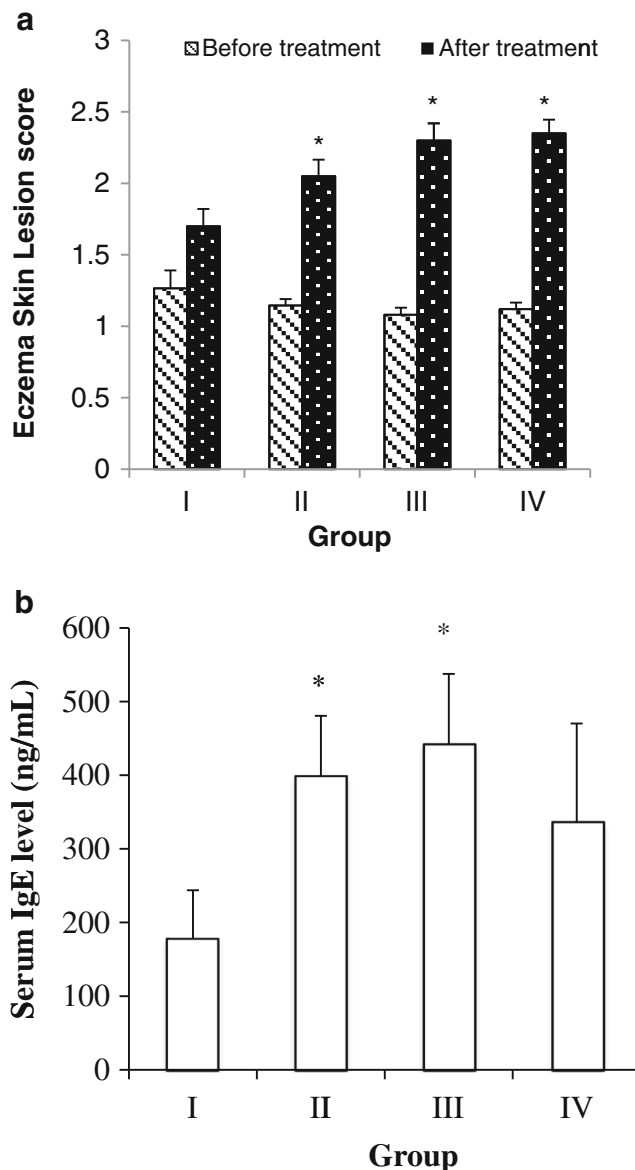


Fig. 5. **a** The skin lesion scores before and after treatment. **b** Serum IgE concentration after treatment. *Indicates significant difference ($p < 0.05$) compared to group I

non-eczematous skin indicated by the solid arrow (Fig. 6a). The dermis of the skin tissue of group I mice also showed an absence of neutrophil infiltration, and the structure of the dermis itself at the eczematous lesion was nearly normal compared with the normal skin. Group II had higher amount of neutrophil infiltration in the dermis as shown by the dark-colored, round in shape organelles, which is indicated by a block arrow in Fig. 6b (17). The epidermis in group II was also thinner but still intact compared with the normal epidermis (solid arrow). However, the dermal structure cannot be identified completely, and most of the dermal components were replaced by adipose tissues, which are colored white. Group III (Fig. 6c) had a condition similar to group II but with a lesser number of neutrophils in the dermis. Group IV mouse skin tissue showed the least membrane intactness (Fig. 6d) but

had a similar number of neutrophils that infiltrated into the dermis as group II.

The histological evaluation of the mice at the end of the treatment revealed minimal inflammation results for the hydrocortisone lyogel treatment (group I) but a higher infiltration of inflammatory mediators in groups II and III. The results also showed continuous inflammation histologically for the Group IV mice that received no treatment. This result suggests that lyogel containing hydrocortisone had an increased inflammatory suppressive effect on eczema compared with commercial cream. The higher concentration of inflammatory mediators in lyogel-free drug treatment compared with group I mice implied that the inflammatory reaction suppression was mainly due to the hydrocortisone, and the vehicle did not contribute to resolving the inflammation. The propylene glycol in the lyogel formulation aids in hydrocortisone permeation into the skin, which supports the result of a lower concentration of inflammatory mediators in the connective tissue of mice receiving hydrocortisone lyogel as eczema treatment. The higher number of neutrophils in the dermis of groups II and IV suggested that these two treatments did not have any inflammatory effects to reduce the number of neutrophils in the dermis.

Dermal Irritancy

Food and Drug Administration (FDA) proposed that propylene glycol is "safe for use in cosmetic products at concentration up to 50%" (18). However, the Cosmetic Ingredient Review (CIR) Expert Panel found conflicting data where only 3 in 16 healthy volunteers show slight skin irritation when propylene glycol (100%) was administered under an occlusive patch, while no reaction was observed up to 50% PG (19). The results of dermal irritancy of lyogel in mice of this study are summarized in Fig. 7.

The resultant indices for erythema for each group are shown in Fig. 7a for each mouse. According to Uttley and Van Abbe (13), values between 0 and 9 indicate that the applied dose would likely not irritate human skin. The result for ear swelling is displayed in Fig. 7b. The left ear of the mice showed a pattern of increasing swelling up to the 4th day, which decreased approaching the 6th day; however, the right ear remained unchanged. The percentage of ear swelling of any day did not exceed 25%, which indicates no or mild skin irritation (20). The left ear of each mouse was applied with 10 mg of hydrocortisone lyogel as specified by the OECD guidelines for dermal irritancy testing. From the results

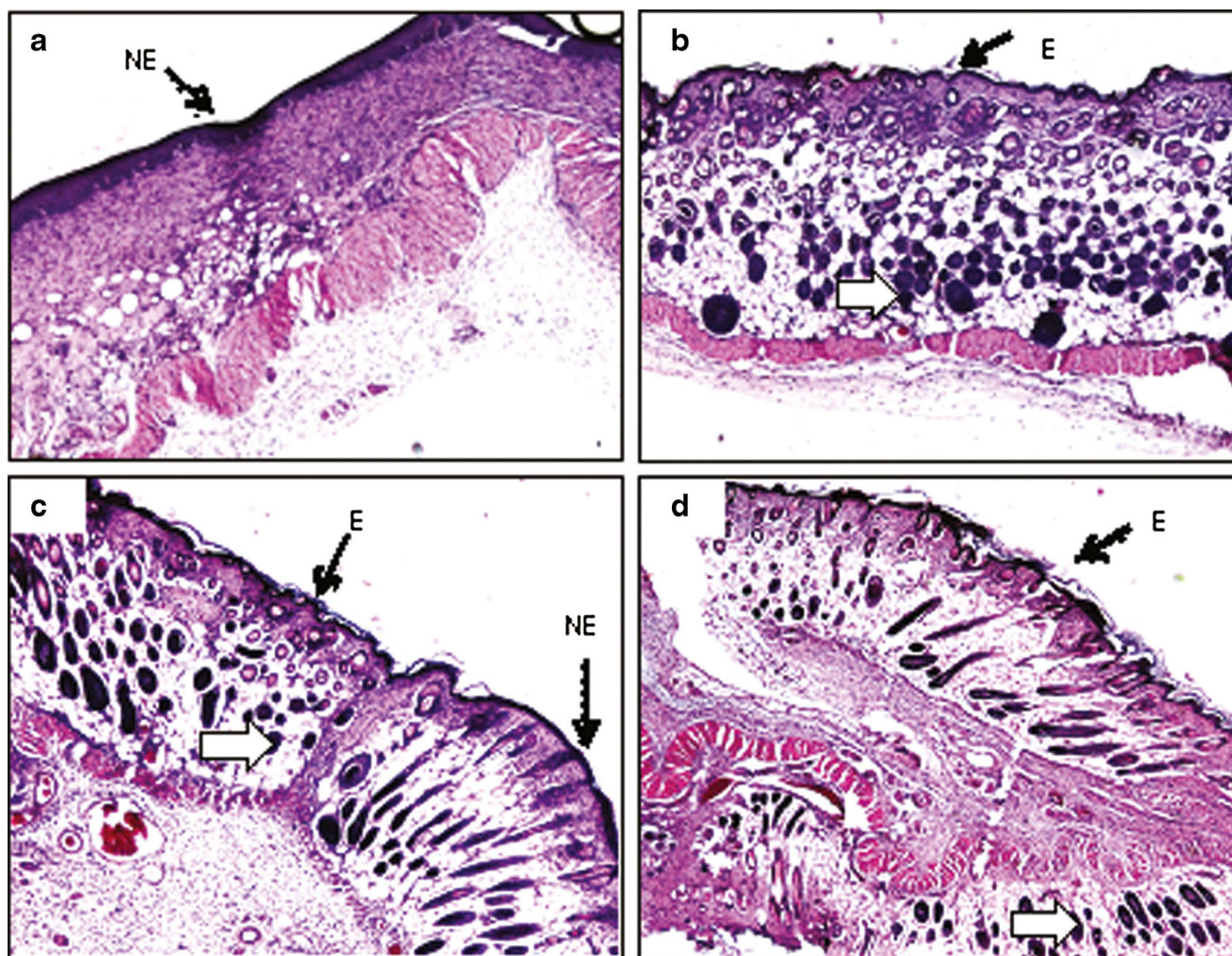


Fig. 6. Histology of **a** group I, **b** group II, **c** group III, and **d** group IV mice skin after 7 days of treatment at $\times 2.5$ magnification. The *arrows* indicate the epidermis of non-eczematous skin part (*NE*) and epidermis of eczematous skin part (*E*), and *block arrows* indicate neutrophils infiltration at dermis

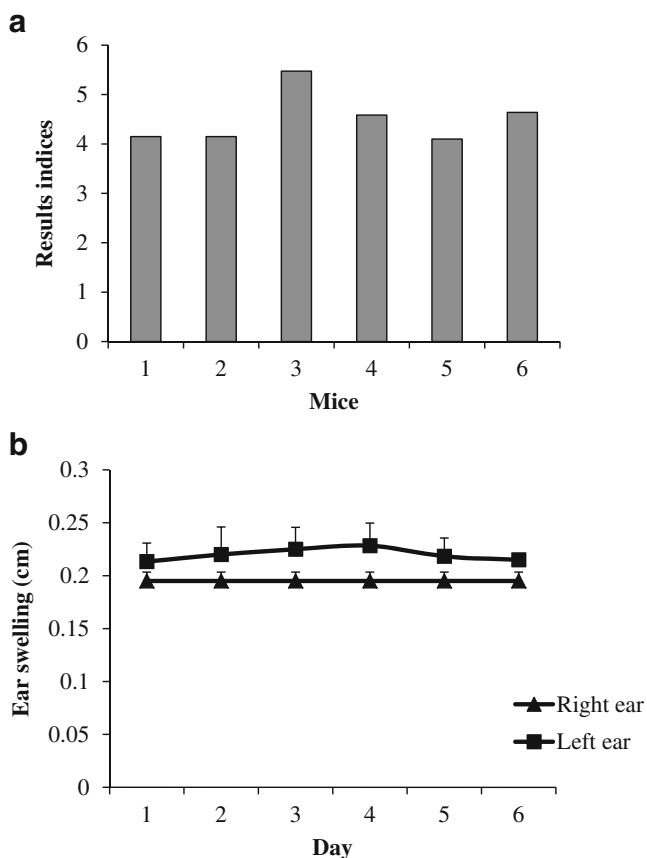


Fig. 7. Dermal irritancy **a** result indices for six mice and **b** ear swelling of left and right ear

obtained for both ear swelling and erythema, the hydrocortisone lyogel likely contains antigenic material with an irritancy potency due to edema and erythema development. However, it is a mild skin irritant as indicated from the resultant indices; scoring between 0 and 9 and <25% ear swelling indicates a lack of skin irritation.

CONCLUSION

The HPMC lyogels containing 1% hydrocortisone appeared clear, soft, and easy to rub on the skin. The lyogel also showed a higher drug release profile compared with commercial hydrocortisone cream; this result may be due to propylene glycol, which acts as a penetration enhancer in lyogel formulations. Similar to the cream, the lyogel exhibited pseudoplastic behavior. From the *in vivo* study, hydrocortisone resolved inflammatory manifestations more effectively within 7 days compared with commercial cream. This resolution was indicated by a reduction in serum IgE levels, decreased neutrophil infiltration, and improved erythema. The dermal irritancy testing revealed that the hydrocortisone lyogel caused minimal irritancy in mice. In conclusion, lyogel is a promising vehicle for delivering hydrocortisone topically as it showed a higher drug release *in vitro* as well as efficacy in resolving the inflammatory reaction associated with eczema compared with commercial cream. As the lyogel exhibits higher drug release, in practice, the application of lyogel should be less frequent in order to minimize side effects.

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