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Cetirizine from Topical Phosphatidylcholine Liposomes: Evaluation of Peripheral Antihistaminic Activity and Systemic Absorption in a Rabbit Model

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ABSTRACT: This study was performed to assess the peripheral H_1-antihistaminic activity and extent of systemic absorption of cetirizine from liposomes applied to the skin. Cetirizine was incorporated into small unilamellar vesicles (SUV) and multilamellar vesicles (MLV) prepared using L-α-phosphatidylcholine, and into Glaxal Base (GB), used as the control. In a randomized, cross-over study, each formulation, containing 10 mg of cetirizine, was applied to depilated areas on the backs of six rabbits (3.08 ± 0.05 kg). Histamine-induced wheal tests and blood sampling were performed before cetirizine application and at designated times for up to 24 h. Compared with the baseline, histamine-induced wheal formation was suppressed by cetirizine in SUV and MLV from 0.5–24 h and by cetirizine in GB from 0.5–8 h, $p \leqslant 0.05$. Maximum wheal suppression by cetirizine in SUV and MLV ranged from $90.6\% \pm 4.9\%$ to $89.0\% \pm 3.8\%$ and $98.0\% \pm 1.3\%$ to $94.0\% \pm 2.3\%$, respectively, from 6 to 8 h. The plasma cetirizine *AUC* of 201 ± 24.2 ng.h/ml from SUV was lower than from PC-MLV, 334.6 ± 65.1 ng.h/ml and from GB, 248.3 ± 34.6 ng.h/ml. After 24 h, the percent of the cetirizine dose remaining on the backs of the rabbits from SUV was lower than from both MLV and GB, $p \leqslant 0.05$. In this model, cetirizine from both SUV and MLV had excellent topical H_1-antihistaminic effects, while systemic exposure to cetirizine from SUV was reduced.

Key words: cetirizine; L-α-phosphatidylcholine; liposomes; antihistamine; skin; rabbits

Introduction

Cetirizine, the active carboxylic acid metabolite of hydroxyzine [1], is a potent second-generation H_1-antihistamine, with anti-inflammatory properties and high specific affinity for histamine H_1-receptors [2–4]. It is widely used to treat symptoms of allergic disease in patients of all ages [5]. It is effective in relieving pruritus, whealing and erythema in urticaria and it reduces pruritus in atopic dermatitis. It may produce somnolence, which is dose-dependent and generally mild [6].

Liposomes are closed vesicles consisting of phospholipid bilayers or lamellas with entrapped aqueous layers and cores that encapsulate active ingredients in their structure [7]. The degree of saturation of the free fatty acid chains in the phospholipids (PL), the principle ingredient of liposome formulations, controls the fluidity of the PL membranes. The addition of cholesterol reduces the fluidity of the membranes above the phase transition temperature, resulting in decreased permeability to hydrophilic solutes and increased stability of the liposomes [8].

Liposomes, as drug delivery systems, have been shown to transport and localize

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hydrophilic, polar drugs into the skin yielding increased therapeutic effects and reduced systemic adverse effects [9,10]. In addition, the application of liposomes moisturizes the skin [11] and reduces the irritation resulting from allergic skin disorders.

The molecular arrangement of the phospholipid and cholesterol in the liposome bilayers is similar to the molecular arrangement of the ceramides, triglycerides, fatty acids and cholesterol in the intercellular bilayers of the stratum corneum with the polar head directed to the aqueous layer and the non-polar tail directed to the lipid layers [12]. This may help the localization and movement of the liposomes within the lipid tail region of the intercellular bilayers of the stratum corneum [11, 13]. The application of antihistamines in ointments or creams to the skin has been used for decades to treat allergic skin disorders, but transdermal absorption into the systemic circulation and toxicity have been reported [14]. It was hypothesized that cetirizine applied to the skin from liposomal formulations, would have a faster onset of action and greater and more prolonged peripheral H_1-antihistaminic activity, accompanied by lower plasma concentrations, than cetirizine from conventional ointments or creams. To test this hypothesis three formulations containing cetirizine: unilamellar vesicles, multilamellar vesicles and the o/w emulsion Glaxal Base, as the control, were evaluated in a randomized cross-over design study in rabbits.

Materials and Methods

Using 406 mg egg L-α-phosphatidylcholine 95% (PC) with a transition temperature (Tc) of −15°C, (personal communication, Avanti Polar Lipids Inc., Alabaster, AL, USA), 61.5 mg cholesterol (Fisher Scientific Co., Fair Lawn, NJ, USA) and 100 ml of 0.2 M phosphate buffer pH 6.5 [15] containing 82 mg cetirizine dihydrochloride (UCB-Pharmaceutical Sector R&D, Braine-L' Alleud, Belgium), small unilamellar vesicles (SUV) were prepared by the ethanol injection method [16] and multilamellar vesicles (MLV) were prepared by the lipid film hydration method [17]. To confirm that the non-medicinal ingredients of liposomes such as the phospholipids and cholesterol did not have any peripheral antihistaminic activity, non-medicated SUV were also prepared. The liposome suspensions were concentrated using an Amicon Ultrafiltration Apparatus, and membrane with a greater than 100 000 M.W. cut-off (Amicon Inc., Beverly, MA, USA). This was accomplished with rapid stirring under nitrogen at 10 psi.

The percent of entrapment of cetirizine was determined by measuring the cetirizine content in the clear filtrate obtained from concentrating and washing the liposome formulations using the Amicon Ultrafiltration Apparatus. The fraction of the amount of cetirizine added initially that would be entrapped within the liposomes was then calculated. Each 1 ml of liposome suspension contained 10 mg of entrapped cetirizine.

The extent of adsorption of cetirizine to the filtration membrane was evaluated by filtration of aqueous solutions of cetirizine at various concentrations. The amount of cetirizine in the aqueous solutions was determined before and after filtration, then any loss by adsorption to the filtration membrane was calculated.

The liposome vesicle sizes in the SUV and MLV formulations were determined using a submicron particle sizer (NICOMP370, Particle Sizing Systems (3–5000 nm), Santa Barbara, CA, USA) and by taking micrographs (TEM) using a transmission electron microscope (Hitachi H-7000, Hitachi Scientific Instruments, Tokyo, Japan). For the submicron particle sizer, volume-weighted Gaussian analysis was used for unimodal distribution and volume-weighted instrument-generated non-Gaussian analysis was used for multi-modal distribution. The run time stopped automatically when a fitting error of 1, or when a Chi-squared value of less than 1 was achieved.

Glaxal Base (GB), a widely used o/w emulsion cream base (Roberts Pharmaceutical Canada Inc., Oakville, ON, Canada) was used as a control formulation. The GB dosage formulation containing 10 mg/g of cetirizine was prepared by dissolving cetirizine in 1 ml of water before levigation into the GB by geometric dilution using a spatula on an ointment slab.

The research approved by the University of Manitoba Fort Garry Campus Protocol Management and Review Committee, was conducted according to current guidelines published by the Canadian Council on Animal Care (CCAC). The study was performed using a randomized crossover design. This study required three formulations each containing 10 mg cetirizine: 1 ml freshly prepared SUV, 1 ml freshly prepared MLV and 1 g GB as a control. Until the animal studies were performed, the freshly prepared liposome batches were stored at 10°C, where stability for up to 2 years has been confirmed [18].

Six New Zealand white rabbits, mean ± SEM weight 3.08 ± 0.05 kg, were studied. Before and between investigations, each rabbit was housed individually in a metal cage with a wire floor to reduce coprophagy. Food and water were supplied *ad libitum*. During initial catheterization and dosing, each rabbit was placed briefly in a restrainer cage (Nalgene, Rochester, NY, USA) then returned to its holding cage. Studies were scheduled 3 or more weeks apart for each animal.

Two days before each study, the hair was cut from a 12 cm × 12 cm area on the back of each rabbit using a hair clipper (Oster A5® size 40, 1/10 mm, Cryotech ™, Fort Madison, IA, USA). One day later, the day before each study, a depilatory (Nair: N.CS: Carter-Wallace, Mississauga, ON, Canada) was applied for 15 min to the 12 cm × 12 cm area on the back and both ears, then removed. To prevent any irritation to the rabbits' skin, the back and ears were thoroughly washed with lukewarm water to ensure all remaining depilatory and hair residues were removed from the hairless areas and proximal hair. The rabbits were dried with clean towels and held in a warm area until completely dry. During the preparation of the rabbits' backs and on each study day before each study commenced, the university veterinarian inspected the rabbits carefully. No visual signs of skin irritation were observed using this procedure.

For blood sampling, a catheter (22G, Critikon Inc., Tampa, FL, USA) was inserted into the ear artery. After 0.5 ml of blood was withdrawn and discarded, a 1.5 ml sample was collected as the pre-dose control. The catheter was flushed with 2 ml of 0.9% sodium chloride (Astra Pharma Inc., Mississauga, ON Canada) followed by 0.2 ml heparin solution (100 IU/ml, Leo Laboratories Canada Ltd, Ajax, ON, Canada).

For dosing, 1 ml of SUV or 1 ml of MLV or 1 g of GB formulation each containing 10 mg cetirizine was applied to a defined 10 cm × 10 cm hairless area on the back of each rabbit. A CCAC approved collar was placed around the neck of each rabbit during the 24 h study to prevent it from dislodging the catheter from the ear artery and from licking the formulations from the hairless back area. Blood sampling was repeated, as previously described for the pre-dose sample, at 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 24 h. The rabbit was returned to its holding cage between sampling intervals of 1 h or longer.

After centrifuging for 15 min at 3000 rpm using Sure Sep-II Separators, (Organon Teknika, Durham, NC, USA) the plasma was stored frozen at −20°C. Later, the samples were thawed and 0.5 ml was used for each analysis. Plasma cetirizine concentrations were analysed using the validated HPLC method developed in our laboratory [19,20].

Cetirizine peripheral antihistaminic activity was assessed by determining the onset and extent of suppression of histamine-induced wheals produced by intradermal injections of 0.05 ml of histamine phosphate, 1.0 mg/ml (Glaxo Smithkline Canada Limited Co., Toronto, ON, Canada). A different site on the 10 cm × 10 cm defined area was used for each skin test. The skin test was performed once before application of the cetirizine formulations to determine the baseline wheal area, then after the application of cetirizine formulations each time a blood sample was taken [19, 20]. Before the first skin test, 1 ml of Evans blue dye (10 mg/ml) (Fisher Scientific, Fairlawn NJ) was injected into the opposite ear vein, to facilitate identification of the histamine-induced wheal circumferences. Wheal circumferences were traced 10 min after each histamine injection, transferred to a transparent paper, and scanned into a computer [19,20]. The histamine skin tests were also performed using non-medicated SUV. The wheal areas were calculated using 5.0 Sigma Scan® Software (Jandel Scientific Corporation, San Rafael, CA, USA). The percent suppression of

the histamine-induced wheals was calculated using the following equation:

$$E = (W_0 - W_t)/W_0 \times 100$$

where E is the percent suppression of the histamine-induced wheals, W_0 is the baseline wheal area and W_t is the wheal area after time (t) of medication application.

After 24 h the liposomes applied to the rabbit's skin would be dehydrated and destabilized [21] so any drug remaining on the defined area on the backs of the rabbits would be no longer entrapped in the liposomes. The amount of the dose remaining on the skin was determined by wiping the defined, treated back area using three gauze sponges wetted with 70% isopropyl alcohol to remove any remaining medication. The gauze sponges were soaked in 100 ml water and stored under refrigeration for 24 h, squeezed to obtain as much of the drug solution as possible, and rinsed two times with 50 ml aliquots of fresh water. The water rinses were combined and the sponges were discarded. The 200 ml solution was filtered, then analysed for cetirizine using the validated HPLC method [19]. The percent of the dose remaining was calculated using the following equation:

$$\text{Percent dose remaining} = (C_{24}/C_{\text{initial}}) \times 100$$

where C_{initial} is the original cetirizine dose applied and C_{24} is the amount of cetirizine remaining after 24 h.

The plasma cetirizine concentrations were plotted versus time, then the area under curve (*AUC*) was calculated using WinNonlin Software (WinNonlin Standard Edition, version 1.1, Scientific Consulting Inc., Apex, NC, USA).

Statistical analysis was performed using the multi-way ANOVA method (split analysis) and Tukey, and Bonferroni methods with the aid of PC- SAS Software (Release 8.02, SAS Institute Inc., Cary, NC, USA). The following analyses were conducted: (a) the histamine-induced wheal areas obtained at each time for each formulation were compared with the pre-dose values, and with values at all times among the formulations; (b) the extent of cetirizine absorbed into the systemic circulation using plasma cetirizine concentrations was compared among the three formulations; and (c) the percentages of the medication remaining on the treated areas of the rabbits' skin were compared among the three formulations. Differences were considered significant at $p \leqslant 0.05$.

Results and Discussion

Liposomes have been used as a carrier system to deliver medications into the skin in order to achieve the therapeutic effect with reduced systemic absorption [22]. By applying cetirizine in liposome formulations to the skin, the peripheral H_1-antihistaminic activity should be retained, potentially improving the therapeutic effects, while absorption into the systemic circulation should be reduced, minimizing the potential for adverse effects.

The SUV liposome formulations had a mean $\pm$ SEM particle size of 165 ± 88 nm, using the submicron particle sizer and 153.5 ± 17.8 nm using TEM, with $92\% \pm 0.2\%$ entrapment of the total amount of cetirizine added. The MLV liposome formulations had a mean $\pm$ SEM particle size of 3405 ± 568 nm, using the submicron particle sizer and 358.3 ± 22.0 nm using TEM with $92.0\% \pm 0.5\%$ entrapment of the total amount of cetirizine added. Using the submicron particle-sizer light-scattering technique method, the mean $\pm$ SEM of particle size distribution of all of the liposomes in the test sample would be determined. However, aggregates would be recorded as individual liposomes, consequently yielding larger mean particle sizes. Using the TEM method, only individual liposomes were identified and measured as observed in the photographs from the mounted samples. The TEM results were similar to those of the submicron particle-sizer results for the SUV, but 10-fold smaller for the MLV prepared in these formulations. The TEM results for the MLV showing the multilamellar characteristics, were considered to be the correct values. The molar ratios of PC:cholesterol:cetirizine per 1 ml of SUV and MLV formulations were 3.2:1:1, calculated using the percent entrapment of the cetirizine.

The concentration of liposome suspensions to determine the extent of cetirizine entrapment by

filtration was valid, as only a negligible amount of the cetirizine content, 0.6%, was adsorbed onto the filtration membrane. A mean cetirizine entrapment of 92% ± 0.4% was achieved at buffer pH 6.5 within the SUV and MLV liposome vesicles prepared for these studies. The high extent of entrapment was probably due to the lipophilicity of the cationic form of cetirizine in the liposomal system ($\log P$=3.2), which was higher than that evaluated in an *n*-octanol/water system ($\log P$=1.12) [23]. This may create a concentration gradient that possibly encourages further entrapment of cetirizine into the liposomes to 92% ± 0.4%.

After application of the non-medicated SUV, the mean ± SEM values of the wheal areas were 1.11 ± 0.03 cm^2, 1.33 ± 0.15 cm^2, 1.46 ± 0.08 cm^2, 1.3 ± 0.08 cm^2 and 1.11 ± 0.13 cm^2 at 0.5, 1, 2, 3 and 4 h, respectively resulting in an overall composite mean ± SEM of 1.25 ± 0.06 cm^2 with a coefficient of variation of 6.8%. There was no significant difference between the pre-dose mean ± SEM wheal areas, 1.18 ± 0.7 cm^2, and the wheal areas at each time interval after application of the non-medicated SUV. Also there was no significant difference between the overall mean wheal area 1.25 ± 0.06 cm^2 and the validated reproducible wheal area, 1.00 ± 0.05 cm^2 obtained as the baseline values before application of the cetirizine containing formulations. These results confirm that phosphatidylcholine and cholesterol did not have any peripheral antihistaminic activity.

Throughout 24 h, the peripheral H_1-antihistaminic activity of cetirizine, measured as the mean ± SEM percent suppression of histamine-induced wheals versus time (Figure 1) from SUV and MLV was superior to GB, $p \leqslant 0.05$.

Cetirizine from both SUV and MLV significantly suppressed the wheal formation for up to 24 h, with maximum wheal suppression from 6 h to 8 h ranging from 90.6% ± 4.9% to 89.0% ± 3.8% and 98.0% ± 1.3% to 94.0% ± 2.3%, respectively. Suppression of the wheals by cetirizine from MLV increased linearly over time from 44.8% ± 9.8% at 0.5 h to 98.0% ± 1.3% at 6 h. In contrast, cetirizine from GB resulted in a maximum suppression of only 70.3% ± 3.8% at 4 h.

The extent of systemic absorption of cetirizine was determined by using mean ± SEM plasma concentrations at selected times after cetirizine application from SUV, MLV and GB, as shown in Figure 2.

Cetirizine plasma concentrations obtained from GB yield a plasma concentration versus time curve similar in shape to those obtained after oral dosing, with a C_{max}, 58.5 ± 5.2 ng/ml at 0.5 h, followed by decreasing concentrations over time as the cetirizine was eliminated, resulting in a mean ± SEM *AUC* of 248.3 ± 34.6 ng.h/ml from 0.5 h to 10 h. This may be due to cetirizine being released rapidly from the GB and absorbed

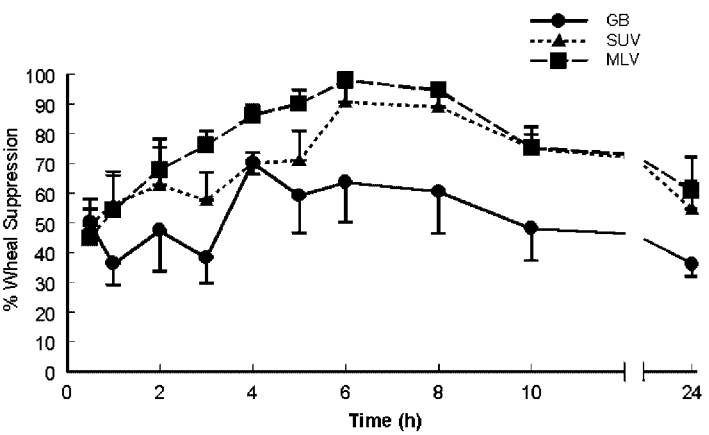

Figure 1. Mean ± SEM percent suppression of histamine-induced wheal formation on the shaved backs of rabbits after the topical application of 10 mg cetirizine from GB, SUV or MLV. GB, Glaxal Base; SUV, small unilamellar vesicles; MLV, multilamellar vesicles

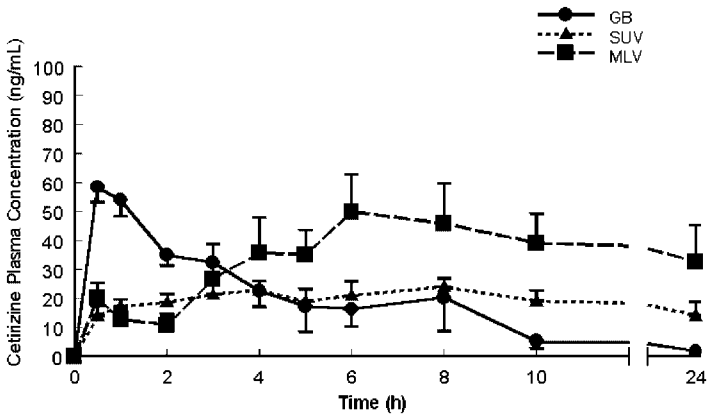

Figure 2. Mean ± SEM cetirizine plasma concentrations after the topical application of 10 mg cetirizine from GB, SUV or MLV on the shaved backs of rabbits. GB, Glaxal Base; SUV, small unilamellar vesicles; MLV, multilamellar vesicles

quickly through the skin into the systemic circulation as a bolus dose. Cetirizine has a relatively small volume of distribution, but would slowly redistribute into the skin and produce the peripheral antihistaminic effects from GB seen in this study, and as shown previously in oral dosing studies in human subjects [20].

Compared with GB, the SUV may act as a controlled release depot in the skin for cetirizine leading to constant cetirizine plasma concentrations over 24 h with *AUC* of 201 ± 24.2 ng.h/ml from 0.5 h to 10 h. This plasma concentration versus time profile may minimize systemic adverse effects, while resulting in a relatively higher peripheral antihistaminic activity for up to 24 h. SUV liposomes may adsorb to the skin surface and penetrate intact through the stratum corneum where modified-release of the medication occurs as reflected from constant cetirizine plasma concentrations over 24 h. In a previous study [19], plasma cetirizine concentrations after a 10 mg cetirizine oral dose to children weighing 25.4 ± 1.9 kg, ranged from 585.6 ng/ml to 1491.6 ng/ml during the 24 h post dose time period. By comparing these results with those obtained after application of SUV containing cetirizine, it is proposed that cetirizine in the liposomes may be concentrated in the skin resulting in a reduction of the histamine-induced wheal reactions [24]. This hypothesis would need to be confirmed by measuring cetirizine concentrations in the skin in a different animal model. It was not possible to perform biopsy studies in the rabbits since the animals could not be euthenized due to the cross-over study design.

From 0.5 to 10 h, the *AUC* of the plasma cetirizine concentrations after application of MLV was 334.6 ± 65.1 ng.h/ml. The increasing cetirizine plasma concentrations after 3 h from MLV was accompanied by increasing wheal suppression. From 0.5 to 3 h, it could be assumed that the peripheral H_1-antihistaminic activity from cetirizine in MLV may be obtained when MLV physically shed the outer lamellas initially releasing some of the medication into the skin. Then after 3 h, MLV could penetrate as oligolamellar vesicles, carrying the drug into the skin, releasing the medication into the skin and the systemic circulation yielding the peripheral antihistaminic effects from MLV. As previously discussed for GB, cetirizine has a relatively small volume of distribution, but would slowly redistribute into the skin and produce the peripheral antihistaminic effects from MLV seen in this study, and as shown previously in oral dosing studies in human subjects [20]. These plasma cetirizine concentrations are still much lower than reported after 10 mg oral doses in children.

This hypothesis may be supported by a previous study conducted by Foldvari *et al.* [25], who found intact unilamellar liposomes (300–500 nm), containing an electron-dense colloidal iron marker, in the dermis of guinea-pigs

using the electron microscope. These investigators also reported that multilamellar liposomes could be found, but less frequently than unilamellar liposomes. In addition the investigators speculated that the unilamellar liposomes (300–500 nm) could penetrate through the 'the lipid channels' of the skin, that is the lipidic material distributed in the intercellular spaces. The investigators also speculated that MLV may shed the outer layers during penetration and could then localize in the skin as uni- or oligolamellar liposomes.

Previous investigators [26–28], found that the sebaceous glands and hair follicles were the major routes of liposome penetration, especially for hydrophilic substances such as isotretinion, carboxyfluorescein and betahistine. In contrast, using hairless mice versus shaved normal mice as experimental models, Honzak *et al*. [29], found that trans-follicular absorption was not a major route of penetration for liposomes into the skin. In their study, the MLV liposomal formulation consisted of phosphatidylcholine plus hydrophosphatidylcholine containing the hydrophilic spin probe GluSL (N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2,3,4,5,6-pentahydroxy-hexane amide). Regardless of the route, in our study, penetration into the skin of cetirizine from all formulations seemed to be rapid, based on the onset time, 0.5 h, of histamine-induced wheal suppression.

Other investigators have also suggested that the liposome formulations might localize entrapped medications in the skin [30–32]. In addition, Patel [33], reported that [^{3}H]-methotrexate entrapped in liposomes was retained in the skin of nude mice to an extent 2–3-fold greater than drug administered in the free state, and was associated with low concentrations of drug in the plasma and with sustained drug effects.

The mean ($\pm$ SEM) percentages of the 10 mg cetirizine dose remaining on the skin at 24 h after the topical application were 9.9% $\pm$ 1.5%, 32% $\pm$ 9.2%, and 17.4% $\pm$ 3.6% for cetirizine from SUV, MLV and GB, respectively. The lowest percent of the cetirizine dose remaining was observed from SUV, $p \leqslant 0.05$. This may be attributed to improved penetration of cetirizine from SUV relative to the other two formulations.

Peripheral H_1-antihistaminic effects evaluated using suppression of the histamine-induced wheal formation were enhanced when cetirizine liposome formulations were applied. The lower plasma cetirizine concentrations from SUV support our hypothesis that these liposomes might localize cetirizine in the skin and might reduce systemic effects. Studies in humans with allergic skin disease are required.

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