

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

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ABSTRACT

Cetirizine, an effective, minimally sedating, second-generation H₁-antihistamine is widely used orally to treat allergic skin disorders. This study was performed to assess the peripheral H₁-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 hydrogenated (HPC), and into Glaxal Base (GB) as the control. In a randomized, crossover study, each formulation, containing 10 mg of cetirizine, was applied to the depilated backs of 6 rabbits (3.08 \pm 0.05 kg). Histamine-induced wheal tests and blood sampling were performed before cetirizine application and at designated times for up to 24 hours afterwards. Compared with baseline, histamine-induced wheal formation was suppressed by cetirizine in SUV only at 24 hours, in MLV from 0.5 to 24 hours, and in GB from 0.5 to 8 hours ($P \leq .05$). Wheal suppression by cetirizine in SUV at 24 hours (91.7% \pm 5.2%) and in MLV from 1 to 24 hours (93.8% \pm 2.2% to 76.2% \pm 6.5%) was greater than in GB (36.5% \pm 7.4% to 60.6% \pm 14.2%) from 1 to 24 hours ($P \leq .05$). Faster onset, as well as greater and more persistent suppression was obtained from cetirizine in MLV. Plasma cetirizine concentrations from MLV (area under the curve [AUC] of 221.2 \pm 42.3 ng.hr/mL) were lower than from GB (AUC of 248.3 \pm 34.6 ng.hr/mL). In this model, cetirizine from MLV had excellent topical H₁-antihistaminic activity, while systemic exposure was reduced, compared with cetirizine from GB.

KEYWORDS: cetirizine, L- α -phosphatidylcholine hydrogenated, liposomes, antihistamine, rabbit's skin.

INTRODUCTION

Cetirizine, the active carboxylic acid metabolite of hydroxyzine, is a potent second-generation H₁-antihistamine, which

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has antiinflammatory properties and high specific affinity for histamine H₁-receptors.¹⁻⁴ 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 the pruritus of atopic dermatitis. The somnolence that sometimes occurs after oral administration is dose dependent, and generally mild.⁶

Liposomes, which are closed vesicles of phospholipid bilayers or lamellae with enclosed aqueous layers and cores, are able to encapsulate hydrophobic and hydrophilic ingredients into their structure.⁷ The degree of saturation of the free fatty acid chains in phospholipids (PL), the principle ingredient of liposome formulations, affects the transition temperature (T_c) of the PL. The transition temperature of L- α -phosphatidylcholine hydrogenated (HPC), 50°C, is much higher than ambient temperatures.⁸

Liposomes, as drug delivery systems, have been shown to transport and localize hydrophilic, polar drugs into the skin yielding increased therapeutic effects and reduced systemic adverse effects.⁹⁻¹⁰ In addition, application of liposomes moisturizes the skin¹¹ and reduces the irritation resulting from allergic skin disorders such as urticaria.

We hypothesized that cetirizine applied to the skin from liposomal formulations would have a faster onset of action and greater and more prolonged peripheral H₁-antihistaminic activity, accompanied by lower plasma concentrations, than cetirizine from conventional ointments or creams. To test this hypothesis, 3 formulations containing cetirizine: unilamellar vesicles, multilamellar vesicles, and the oil/water (o/w) emulsion Glaxal Base (GB), as the control, (Roberts Pharmaceutical Canada Inc, Oakville, ON, Canada) were evaluated in a randomized crossover design study in rabbits.

MATERIALS AND METHODS

Using 407 mg L- α -phosphatidylcholine 95% (hydrogenated-egg) (HPC) (Avanti Polar Lipids Inc, Alabaster, AL), 61.5 mg cholesterol (Fisher Scientific Co, Fair Lawn, NJ), and 100 mL of 0.2 M phosphate buffer pH 6.5,¹² multilamellar vesicles (MLV) containing 82 mg cetirizine dihydrochloride (UCB-Pharmaceutical Sector R&D, Braine-L'Alleud, Belgium) were prepared by the lipid film hydration method¹³ at 55°C. The

liposome suspensions were concentrated to 10 mL using an Amicon Ultrafiltration Apparatus and membrane with a greater than 100 000 molecular weight cut-off (Amicon Inc, Beverly, MA). This was accomplished with rapid stirring under nitrogen at 10 psi. Small unilamellar vesicles (SUV) of cetirizine were prepared by extruding 10 mL concentrated, freshly prepared HPC-MLV 11 times through polycarbonate membranes of 200 μm followed by an additional 11 times through polycarbonate membranes of 0.03 μm (Avanti Polar Lipids) using a mini-extruder (Avanti Polar Lipids). The percentage of entrapment of cetirizine was determined by measuring the cetirizine content in the clear filtrate obtained from concentrating the liposome formulations using the Amicon Ultrafiltration Apparatus. The fraction of the amount of the 82 mg of cetirizine dihydrochloride initially added, which would be entrapped within the liposomes, was then calculated. 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; and any loss by adsorption to the filtration membrane was calculated.

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

GB, a widely used o/w emulsion cream base 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 animal research study, 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 3 formulations: freshly prepared HPC-SUV, freshly prepared HPC-MLV, and GB containing cetirizine as a control. The HPC transition temperature, 50°C, is well above ambient temperature, and HPC liposome formulations are stable for up to 2 years even when stored above 25°C¹⁴; however, all formulations were stored in tightly sealed containers at 10°C in a refrigerator until the day of in vivo evaluation.

Six New Zealand white rabbits, mean \pm SEM weight 3.08 \pm 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) then returned to its own 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 \times 12-cm area on the back of each rabbit using a hair clipper (Oster A5 size 40, 1/10 mm, Cryotech, Fort Madison, IA). The day before each study, a depilatory (Nair: N.CS: Carter-Wallace, Mississauga, ON, Canada) was applied for 15 minutes to the 12-cm \times 12-cm area on the back and both ears; then was 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 adjacent hair. The rabbits were dried with a clean towel 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.

For blood sampling, a catheter (22G, Critikon Inc, Tampa, FL) 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 predose control and placed in a vacutainer (Baxter Healthcare Co, Valencia, CA) with no additives. The catheter was flushed with 2 mL of 0.9% sodium chloride (AstraZeneca Canada Inc, Mississauga, ON, Canada) followed by 0.2 mL of 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, or 1 mL of non-medicated SUV¹⁵ was applied to a defined 10-cm \times 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-hour 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 predose sample, at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 24 hours. The rabbit was returned to its holding cage between sampling intervals of 1 hour or longer.

Using Sure Sep-II separators (Organon Teknika, Durham, NC), the blood samples were centrifuged for 15 minutes at 3000 rpm, and the plasma was separated and frozen at -20°C. Plasma cetirizine concentrations were analyzed using the validated high-performance liquid chromatography (HPLC) method developed in our laboratory.¹⁶

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 in 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. Before the first skin test, 1 mL of Evans blue dye (10 mg/mL) (Fisher Scientific) was injected into the opposite ear vein to facilitate identification of the histamine-induced wheal circumferences. Wheal circumferences were traced 10 minutes after each histamine injection, transferred to a transparent paper, and scanned into a computer.¹⁶ The wheal areas were calculated using 5.0 Sigma Scan Software (Jandel Scientific Corporation, San Rafael, CA). The percentage suppression of the histamine-induced wheals was calculated using the following equation:

$$E = (W_0 - W_t)/W_0 \times 100 \quad (1)$$

where E is the percentage 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.

It was expected that after 24 hours the liposomes applied to the rabbits' skin would probably be dehydrated and destabilized,¹⁷ so any drug remaining on the defined area on the backs of the rabbits would no longer be entrapped in the liposomes. The amount of the dose remaining on the skin was determined by wiping the defined, treated back area using 3 gauze sponges wetted with 70% isopropyl alcohol to remove any remaining medication. The gauze sponges were soaked in 100 mL of water and stored under refrigeration for 24 hours, squeezed to obtain as much drug solution as possible, and rinsed 2 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 analyzed for cetirizine using the validated HPLC method.¹⁶ The percentage of dose remaining was calculated using the following equation:

$$\text{Percentage Dose Remaining} = (C_{24}/C_{\text{initial}}) \times 100, \quad (2)$$

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

Following analysis of the blood samples, the plasma cetirizine concentrations were plotted vs time; then the AUC was calculated using WinNonlin Software (WinNonlin Standard Edition, Version 1.1, Scientific Consulting Inc, Apex, NC).

Statistical analysis was performed using multiway analysis of variance (ANOVA) method (split analysis) and Tukey and Bonferroni methods with the aid of PC-SAS Software (Release 8.02, SAS Institute Inc, Cary, NC). The following analyses were conducted: (1) the histamine-induced wheal areas obtained at each time for each formulation were compared with the predose values, and with values at all times among the formulations; (2) the extent of cetirizine absorbed

into the systemic circulation using plasma cetirizine concentrations was compared among the 3 formulations; and (3) the percentages of the medication remaining on the treated skin areas were compared among the 3 formulations. Differences were considered significant at $P \leq .05$.

RESULTS

The SUV liposome formulations had a mean ± standard error of the mean (SEM) particle size of $1.93 \pm .031 \mu\text{m}$ using the submicron particle sizer and $40 \pm 4 \text{ nm}$ using TEM, with $92.8\% \pm 0.3\%$ entrapment of the total amount of cetirizine added. The MLV liposome formulations had a mean ± SEM particle size of $20.2 \pm 1.0 \mu\text{m}$ using the submicron particle sizer and $296 \pm 27 \text{ nm}$ using TEM, with $89.7\% \pm 0.4\%$ entrapment of the total amount of cetirizine added. The molar ratios of HPC:cholesterol:cetirizine per 1 mL of SUV and MLV formulations were 3.0:1:1 and 3.3:1:1, respectively, calculated using the percentage entrapment of the cetirizine.

Cetirizine peripheral H₁-antihistaminic activity, measured as mean (± SEM) percentage suppression of histamine-induced wheals vs time is shown in Figure 1.

Compared with the predose wheal areas, a rapid onset of H₁-

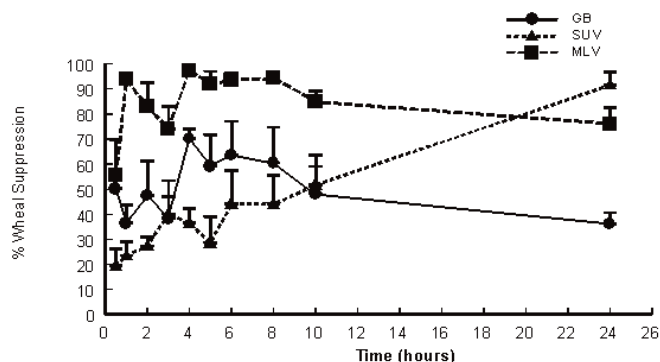


Figure 1. Mean (± SEM) percentage suppression of histamine-induced wheal formation on the depilated backs of rabbits after the topical application of 10 mg cetirizine from GB, SUV, or MLV. GB indicates Glaxal Base; SUV, small unilamellar vesicles; and MLV, multilamellar vesicles.

antihistaminic activity was achieved from cetirizine in HPC-MLV. After 1 hour, the suppression was $93.8\% \pm 2.2\%$ compared with HPC-SUV, $23.6\% \pm 5.5\%$, and GB, $36.5\% \pm 7.4\%$, $P \leq .05$. Compared with predose results, significant wheal suppression from cetirizine in HPC-SUV was achieved only at 24 hours, $91.7\% \pm 5.2\%$, while in HPC-MLV suppression was significant from 0.5 to 24 hours, and in GB from 0.5 to 8 hours, $P \leq .05$.

Cetirizine from HPC-MLV yielded a prolonged maximum suppression of $97.5\% \pm 1.4\%$ to $94.4\% \pm 1.7\%$ from 4 hours to 8 hours. Cetirizine from GB yielded a maximum suppres-

sion of only $70.3\% \pm 3.8\%$, at 4 hours.

After 24 hours, the peripheral H_1 -antihistaminic activity of cetirizine from HPC-SUV, $91.7\% \pm 5.2\%$, was superior to GB, 36.3 ± 4.3 , $P \leq .05$. Overall, the peripheral H_1 -antihistamine activity of cetirizine from HPC-MLV was superior and prolonged from 4 to 8 hours, ranging from $97.5\% \pm 1.4\%$ to $94.9\% \pm 1.7\%$ compared with HPC-SUV, $36.5\% \pm 5.9\%$ to $44.1\% \pm 11.3\%$, and GB, $70.3\% \pm 3.8\%$ to $60.6\% \pm 14.2\%$.

The extent of systemic absorption of cetirizine was determined by evaluating the mean plasma cetirizine concentrations at selected times after cetirizine application, as shown in Figure 2.

The AUC of plasma cetirizine concentrations from 0.5 to 24

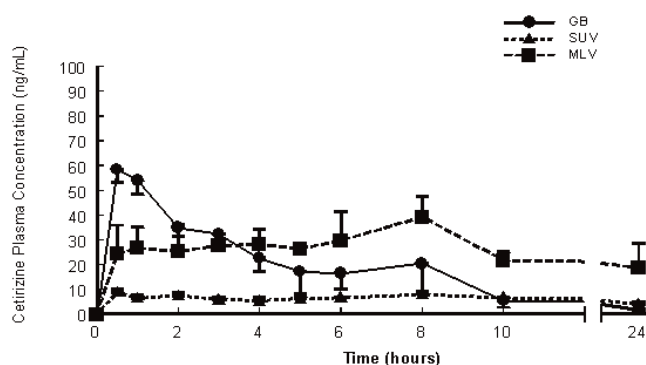


Figure 2. Mean (\pm SEM) cetirizine plasma concentrations after the topical application of 10 mg cetirizine from GB, SUV, or MLV on the depilated backs of rabbits. GB indicates Glaxal Base; SUV, small unilamellar vesicles; and MLV, multilamellar vesicles.

hours after HPC-SUV, 67 ± 5.2 ng.hr/mL, was significantly lower than after GB application, AUC of 248.3 ± 34.6 ng.hr/mL, $P \leq .05$. The mean (\pm SEM) plasma cetirizine concentrations from 0.5 to 8 hours after HPC-SUV were lower but not significantly different compared with cetirizine from HPC-MLV. From 0.5 to 2 hours, the mean plasma cetirizine concentrations from HPC-MLV, 24.3 ± 11.7 ng/mL to 25.3 ± 10.8 ng/mL, were significantly lower than from GB, 35.0 ± 3.7 ng/mL to 58.5 ± 5.2 ng/mL, $P \leq .05$. The AUC of HPC-MLV was 221.2 ± 42.3 ng.hr/mL.

The mean (\pm SEM) percentage of cetirizine dose remaining on the skin at 24 hours after the topical application of 10 mg of cetirizine from HPC-SUV, $5.9\% \pm 0.7\%$, was significantly lower compared with HPC-MLV, $19.2\% \pm 4\%$, and with GB, $17.4\% \pm 3.6\%$, $P \leq .05$.

DISCUSSION

In recent studies, various medications have been incorporated into liposomes for topical delivery.^{7-11,18} Cetirizine, a

potent second-generation H_1 -antihistamine, is effective for the treatment of allergic skin disorders but is minimally sedating when given orally. It was hypothesized that by administering cetirizine in liposome formulations to the skin, the peripheral H_1 -antihistaminic activity would be retained, while absorption into the systemic circulation would be reduced, potentially reducing even the minimal sedative adverse effects. In both the SUV and MLV liposomes prepared for these studies, a very high percentage of the amount of cetirizine incorporated into the formulations was entrapped within the liposome vesicles. The SUV and MLV liposome formulations, using phosphate buffer pH 6.5, yielded a mean entrapment of $92.8\% \pm 0.3\%$ and $89.7\% \pm 0.4\%$, respectively, of the total amount of 82 mg cetirizine added during preparation of the liposomes. The concentration of liposome suspensions to determine the extent of cetirizine entrapment by ultrafiltration was valid as only a negligible amount of the nonencapsulated cetirizine content, 0.6% , was adsorbed from aqueous solutions onto the filtration membrane. The high extent of entrapment into the lipid vesicles 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 ($\log P = 1.12$).¹⁹ This may create a concentration gradient that possibly encourages further entrapment of cetirizine into the liposomes to the $89.7\% \pm 0.4\%$ and to $92.8\% \pm 0.3\%$ in MLV and SUV, respectively.

The mean \pm SEM particle sizes of the liposomes, determined using the submicron particle sizer method for SUV and MLV, were $1.93 \pm .031$ μm and 20.2 ± 1.0 μm , respectively. This was larger than that found by measuring the liposomes from the TEM, where SUV and MLV resulted in mean \pm SEM sizes of 40 ± 4 nm and 296 ± 27 nm, respectively. 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 was 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 sample (not shown). The TEM results more accurately represent the true sizes of the SUV and MLV prepared in these formulations.

To confirm that the nonmedicinal ingredients of the liposomes, the phospholipids and cholesterol, did not have any peripheral antihistaminic activity, the histamine-induced wheal skin test was performed in a previous study¹⁵ after application of nonmedicated SUV prepared using L- α -phosphatidylcholine (PC). The mean \pm SEM values of the wheal areas were 1.11 ± 0.03 cm², 1.33 ± 0.15 cm², 1.46 ± 0.08 cm², 1.3 ± 0.08 cm², and 1.11 ± 0.13 cm² at 0.5, 1, 2, 3, and 4 hours, respectively, resulting in an overall composite mean \pm SEM of 1.25 ± 0.06 cm² with a coefficient of variation of

6.8%. There was no significant difference between the pre-dose mean \pm SEM wheal areas, $1.18 \pm 0.7 \text{ cm}^2$, and the wheal areas at each time interval after application of the nonmedicated SUV. Also, there was no significant difference between the overall mean wheal area $1.25 \pm 0.06 \text{ cm}^2$ and the validated reproducible wheal area, $1.00 \pm 0.05 \text{ cm}^2$ obtained as the baseline values before application of the cetirizine-containing formulations. These results confirm that phosphatidylcholine and cholesterol do not have any antihistaminic activity. These results can be applied to the current studies with HPC as there is virtually no difference in the chemical structure between both PC and HPC. In addition, phospholipids have never been reported to exhibit any antihistaminic activity.

From 0.5 to 24 hours, an increasing linear relationship was observed between the mean percentage suppression of histamine-induced wheal by cetirizine from HPC-SUV vs time. The cetirizine plasma concentrations from HPC-SUV remained relatively low and consistent compared with HPC-MLV and GB, perhaps indicating a zero-order release of medication into the systemic circulation. In contrast, the drug concentrations at the receptor site in the skin may have increased with time resulting in a steady increase in the wheal suppression. These results need confirmation by performing additional skin tests and taking additional blood samples between 10 and 24 hours, and further testing after 24 hours to determine the duration of the efficacy. It might also be possible to determine skin concentrations of cetirizine in a different animal model.

The MLV liposome formulations yielded relatively low consistent plasma cetirizine concentrations in conjunction with rapid onset and duration of significant peripheral H_1 -antihistaminic effects, as monitored by suppression of the histamine-induced wheals, compared with both HPC-SUV and GB. In a previous study,²⁰ after a 10-mg cetirizine oral dose to children ($25.4 \pm 1.9 \text{ kg}$), plasma cetirizine concentrations ranged from 585.6 to 1491.6 ng/mL during the 24-hour post-dose time period. By comparing these plasma cetirizine concentrations with those obtained after application of liposomes containing 10 mg 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.²¹ This hypothesis may be supported by a previous study conducted by Foldvari et al,²² who found intact unilamellar liposomes (300-500nm), containing an electron-dense colloidal iron marker, in the dermis of guinea pigs using the electron microscope. These investigators also reported that multilamellar liposomes were found less frequently than unilamellar liposomes. In addition, the investigators speculated that the unilamellar liposomes (300-500nm) could penetrate through the "lipid channels" of the skin (ie, 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.

Depending on the above explanations, it could be assumed that a rapid onset of peripheral H_1 -antihistaminic activity obtained from cetirizine in MLV may be obtained when MLV physically shed the outer layers, initially releasing some of the medication into the skin. Then MLV could penetrate as oligolamellar vesicles, carrying the drug into the skin and releasing the medication consistently over the next 24 hours as the skin endogenous phospholipases sequentially degrade the remaining lamellae. The SUV, in contrast, may penetrate the skin as intact liposomes and then release the medication in a modified-release process as the skin endogenous phospholipases degrade the lamellae during the ensuing 24 hours. This possible mechanism of liposome penetration would explain the low concentrations of cetirizine in the systemic circulation from the 2 liposome formulations. 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 crossover study design.

Cetirizine plasma concentrations obtained from GB, when plotted vs time as shown in Figure 2, yield a plasma concentration vs time curve similar in shape to those obtained after oral dosing, with a C_{max} at 0.5 hours, followed by decreasing concentrations over time as the cetirizine was eliminated. Cetirizine plasma concentrations from GB were much lower than those obtained from a 10-mg oral dose²⁰ but higher than those obtained from HPC-SUV and HPC-MLV. This finding may be due to cetirizine being released rapidly from the GB and absorbed 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.¹⁶

The HPC-SUV resulted in the lowest percentage of the cetirizine dose remaining on the skin when compared with the other 2 formulations, $P \leq .05$. This finding may be attributed to improved penetration and concentration of the drug into the skin from SUV but delayed release when compared with the other 2 formulations. The relatively low plasma cetirizine concentrations from HPC-SUV are therefore not due to a lack of absorption of the medication. The increasing concentrations of cetirizine in the skin from HPC-SUV hypothesis is supported by the linear increase of peripheral H_1 -antihistaminic activity over time from 0.5 hours up to 24 hours.

The higher and faster peripheral H_1 -antihistamine activity as well as higher plasma concentrations of cetirizine from HPC-MLV relative to HPC-SUV are similar to the results of Egbaria et al.^{23,24} These researchers found that MLV lipo-

somes of cyclosporin prepared using different phospholipids produced a deeper drug penetration in the skin strata of humans and hairless mice than that found from SUV. After penetration, HPC-SUV may retain the cetirizine until the liposome bilayer membrane is degraded by endogenous phospholipases,²⁵⁻²⁷ resulting in the slow release of cetirizine over time into the skin, which provides increasing wheal suppression but persistently low plasma concentrations. These results need to be confirmed by measuring cetirizine concentration in the skin and detecting the intact liposomes in the skin.

Peripheral H₁-antihistaminic effects evaluated using suppression of the histamine-induced wheal formation was enhanced from cetirizine MLV liposome formulations compared with cetirizine from SUV and GB. The accompanying lower plasma cetirizine concentrations from liposome formulations, compared with those obtained following GB and when compared with those measured after oral administration to humans,^{16,20} support our hypothesis that liposomes might localize cetirizine in the skin and might possibly reduce the incidence of any adverse effects of the medication. Further studies in humans with allergic skin disease are required to confirm the activity of the HPC-MLV formulation.

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