AAPS PharmSci 2003; 5 (4) Article 28 (http://www.aapspharmsci.org).

Hydroxyzine From Topical Phospholipid Liposomal Formulations: Evaluation of Peripheral Antihistaminic Activity and Systemic Absorption in a Rabbit Model

Submitted: July 16, 2003; Accepted: September 2, 2003; Published: November 5, 2003

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ABSTRACT

Hydroxyzine, an effective but sedating H_1-antihistamine is given orally to treat allergic skin disorders. This study was performed to assess the peripheral H_1-antihistaminic activity and extent of systemic absorption of hydroxyzine from liposomes applied to the skin. Using L-α-phosphatidylcholine (PC), small unilamellar vesicles (SUVs) and multilamellar vesicles (MLVs) containing hydroxyzine were prepared. Hydroxyzine in Glaxal Base (GB) was used as the control. Using a randomized, crossover design, each formulation, containing 10 mg of hydroxyzine, was applied to the shaved backs of 6 rabbits (3.08 ± 0.05 kg). Histamine-induced wheal tests and blood sampling were performed at designated time intervals up to 24 hours. Compared with baseline, hydroxyzine from all formulations significantly suppressed histamine-induced wheal formation by 75% to 95% for up to 24 hours. Mean maximum suppression, 85% to 94%, occurred from 2 to 6 hours, with no differences among the formulations. The areas of plasma hydroxyzine concentration versus time area under the curve (AUCs) from PC-SUV and PC-MLV, 80.1 ± 20.8 and 78.4 ± 33.9 ng/mL/h, respectively, were lower than that from GB, 492 ± 141 ng/mL/h ($P \leq .05$) over 24 hours. Plasma concentrations of cetirizine arising in-vivo as the active metabolite of hydroxyzine, from PC-SUV, PC-MLV, and GB, were similar with AUCs of 765 ± 50, 1035 ± 202, and 957 ± 227 ng/mL/h, respectively ($P \leq .05$). Only 0.02% to 0.06% of the initial hydroxyzine dose remained on the skin after 24 hours. In this model, hydroxyzine from SUV and MLV had excellent topical H_1-antihistaminic activity, and minimal systemic exposure occurred. Cetirizine formed in-vivo contributed to some of H_1-antihistaminic activity.

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KEYWORDS: hydroxyzine, L-α-phosphatidylcholine, liposomes, antihistamine, skin, rabbit

INTRODUCTION

Hydroxyzine, a piperazine-class H_1-antihistamine, is effective in the treatment of urticaria and other allergic skin disorders in which histamine plays a role. After conventional oral administration, the major adverse effects of hydroxyzine include central nervous system (CNS) sedation and impairment of cognitive and psychomotor function, and anticholinergic effects including dry mouth and urinary retention.[1]

Topical application of first-generation H_1-antihistamines in ointments and creams for treatment of symptoms of allergic skin disorders has been in use for many years; however, considerable systemic absorption occurs, potentially leading to systemic side effects.[2]

Drug carrier systems that deliver medication into the skin to provide therapeutic effects with reduced systemic absorption and reduced adverse effects are currently under investigation. One system being studied is drug encapsulation in liposomes, which have the capability of overcoming some of the well-known problems in drug delivery to the skin. Liposomes potentially enhance drug penetration into the stratum corneum,[3-9] maintain drug release in the epidermis,[10-12] and localize the drug within the dermo-epidermal layers, while reducing the amount of drug absorbed into the systemic circulation.[13,14] Liposomes also form occlusive films, which lead to increased skin hydration and to increased drug penetration into the stratum corneum.[15,16] Moreover, as they are composed of phospholipids, a natural

component of the cell membranes in skin, they act as nonirritating moisturizing agents.

We hypothesized that, because of entrapment of the medication in the liposome phospholipid vesicles, the first-generation H_1-antihistamine hydroxyzine would have greater and more prolonged peripheral H_1-activity when applied to the skin in liposomal formulations than when applied in a conventional emollient skin cream. We also hypothesized that topical administration of this potentially sedating H_1-antihistamine in liposome formulations would provide prompt onset of action and prolonged duration of action because effective concentrations in the skin would be maintained, while systemic serum concentrations would be low. To test this hypothesis, 3 formulations containing hydroxyzine: small unilamellar vesicles (SUVs), multilamellar vesicles (MLVs), and Glaxal Base (GB) (Roberts Pharmaceutical Canada, Oakville, ON, Canada) as the control, were evaluated in a randomized crossover design study in rabbits.

MATERIALS AND METHODS

Using egg L-α-phosphatidylcholine 95% (PC) (Avanti Polar Lipids, Alabaster, AL) and 0.2M phosphate buffer, pH 6.5, SUVs were prepared by the ethanol injection method[17] and MLVs were prepared by the lipid film hydration method.[18] The liposome suspensions were concentrated using an Amicon ultrafiltration apparatus, and membrane with a greater than 100 000 molecular weight (MW) cutoff (Amicon, Beverly, MA). This was accomplished with rapid stirring under nitrogen at 10 psi. The molar ratio of PC to cholesterol (Fisher Scientific, Fair Lawn, NJ) to hydroxyzine (Sigma Chemical, St Louis, MO) per 1 mL of SUV and MLV formulations was 3.5:0.86:1 and 3.2:1:1, respectively.

The liposome vesicle sizes in the SUV and MLV formulations were determined using the NICOMP 370 Submicron Particle Sizer (Nicomp, Santa Barbara, CA). 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. The percentage entrapment of hydroxyzine was determined by measuring the hydroxyzine content in the clear filtrate obtained from concentrating the liposome formulations using the Amicon ultrafiltration apparatus.[17,18] The fraction of the amount of hydroxyzine initially added that would be entrapped within the vesicles was then calculated. The SUV liposome formulations had a mean ± SEM particle size of 265 ± 198 nm, with 86% ± 0.5% entrapment of the total amount of hydroxyzine added. The MLV liposome formulations had a mean ± SEM particle size of 4.87 ± 0.65 μm, with 94.3% ± 0.4% entrapment of the total amount of hydroxyzine added.

The PC transition temperature (-15°C) is well below ambient temperature, and liposome formulations become less stable when stored above 25°C.[19] The freshly prepared liposome batches were stored at 10°C, at which stability for up to 2 years has been confirmed,[19] until the rabbit studies were performed.

The GB cream formulation, used as the control, was prepared by levigating 10 mg of hydroxyzine with a few drops of water before mixing 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).[20] 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) and then returned to its own holding cage. Studies were scheduled 3 or more weeks apart for each animal.

Two days before each study, a 12-cm × 12-cm area on the back of each rabbit was shaved using an electric razor. One day before each study, a depilatory was applied for 15 minutes to the shaved area on the back and to both ears, then thoroughly washed off, to ensure complete removal of the hair.

For blood sampling, a catheter (22G, Critikon, 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, 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 heparin solution (100 IU/mL, Leo Laboratories Canada, Ajax, ON, Canada).

For dosing, 1 mL of SUV or MLV or 1 g of GB formulation, each containing 10 mg hydroxyzine was applied to the defined area on the rabbit's back. A CCAC-approved collar was placed around the neck of each rabbit during the 24-hour study to prevent it from lick-

ing its back or dislodging the catheter from the ear artery. The 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 or more hours.

After centrifuging for 15 minutes at 3000 rpm with the aid of a Sure Sep-II separator (Organon Teknika, Durham, NC), the plasma was stored frozen at -20°C. Later, the samples were thawed, and 0.5 mL was used for each analysis. Plasma hydroxyzine and cetirizine concentrations were analyzed using the validated high performance liquid chromatography (HPLC) methods developed in our laboratory.[21,22]

Each time a blood sample was taken, hydroxyzine activity was assessed using an intradermal injection of 0.05 mL of histamine phosphate, 1.0 mg/mL (Glaxo SmithKline Canada,Toronto, ON, Canada). A different site on the designated area to which the hydroxyzine formulation was applied was used for each test. 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. The predose wheal area and wheal areas at each time interval were traced after 10 minutes.[23] The traced wheals, transferred to a transparent paper, were scanned into a computer, and the wheal areas were calculated using 5.0 Sigma Scan software (Jandel Scientific, San Rafael, CA). The percentage suppression of the histamine-induced wheals was used as an indication of peripheral H_1-antihistaminic activity, calculated using Equation 1.

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

where E is the efficacy of the medication; W_0 is the baseline wheal area; and W_t is the wheal area after time (t) of medication application.

After 24 hours, the amount of the dose remaining on the skin was determined by wiping the defined, treated back area using 1 or more gauze sponges wetted with isopropyl alcohol, 70%, to remove any remaining medication. The gauze sponges were soaked in water, squeezed to remove as much of the solvent as possible, then removed. The turbid aqueous solutions were filtered to remove any traces of PC and then were analyzed for hydroxyzine using the validated HPLC method.[21] The percentage of dose remaining was calculated using Equation 2.

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

where H_{initial} is the original hydroxyzine dose applied and H_{24} is the amount of hydroxyzine remaining after 24 hours.

Statistical analysis was performed using multiway ANOVA (split analysis) and Tukey and Bonferroni methods with the aid of PC-SAS software (Release 8.02, SAS Institute, Cary, NC). The following statistical analyses of the data 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 medication absorbed into the systemic circulation using plasma hydroxyzine and cetirizine concentrations was compared among the 3 formulations; and (3) the percentage of the medication remaining on the backs of the rabbits was compared among the 3 formulations. Differences were considered significant at $P \leq .05$.

RESULTS

Hydroxyzine peripheral H_1-antihistaminic activity over time is shown in **Figure 1**, as mean percentage suppression of histamine-induced wheals. In comparison with the predose wheal areas, all hydroxyzine formulations significantly suppressed wheal formation for up to 24 hours ($P \leq .05$). Onset of suppression was rapid, with about 75% suppression occurring as early as 0.5 hour. Maximum suppression, 85% ± 5.6% to 94% ± 5%, was present from 2 to 6 hours. There was no significant difference in activity among the formulations PC-SUV, PC-MLV, and GB (control) containing hydroxyzine, for up to 24 hours ($P \leq .05$). However, hydroxyzine from PC-SUV and PC-MLV resulted in greater wheal suppression (85.8% ± 5.6% to 94.5% ± 2.6%) from 2 to 6 hours, than hydroxyzine from GB (73.3% ± 7.5% to 76.1% ± 11.0%).

The extent of systemic absorption of hydroxyzine was determined from the mean plasma hydroxyzine concentrations measured at the preselected times after hydroxyzine application, as shown in **Figure 2**. The mean plasma hydroxyzine concentrations after PC-SUV and PC-MLV application were similar to each other, and significantly lower than those after GB application for up to 24 hours ($P \leq .05$). Compared with both PC-SUV and PC-MLV formulations, which resulted in mean plasma concentrations of only 12.5 ± 12.1 to 0.9 ± 0.4 ng/mL, with an AUC of 80.1 ± 20.8 ng.h/mL, and

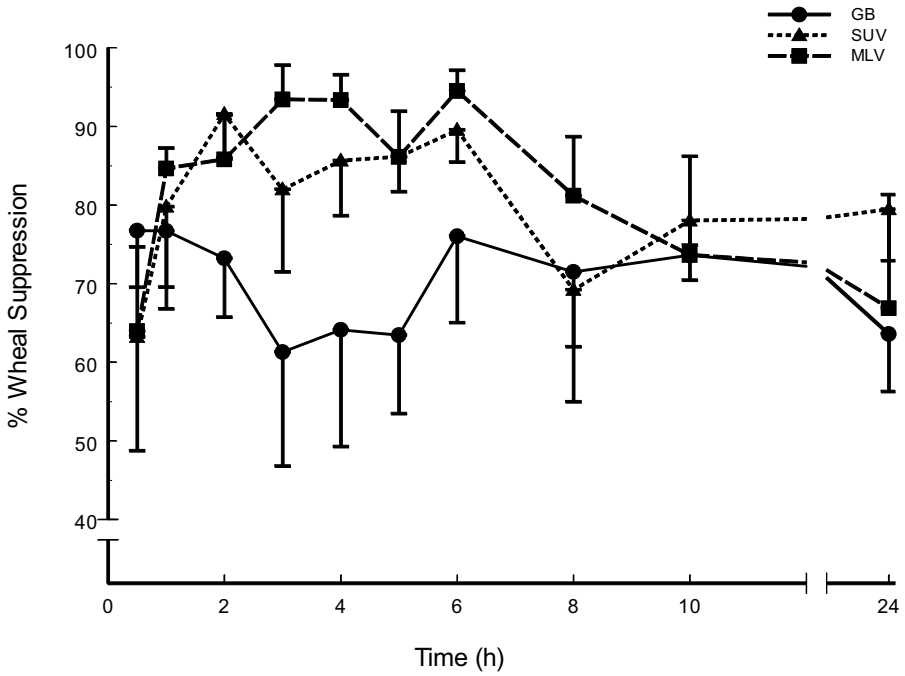

Figure 1. Mean (± SEM) percentage suppression of histamine-induced wheal formation on the shaved backs of rabbits after the topical application of 10 mg hydroxyzine from GB or SUV or MLV.

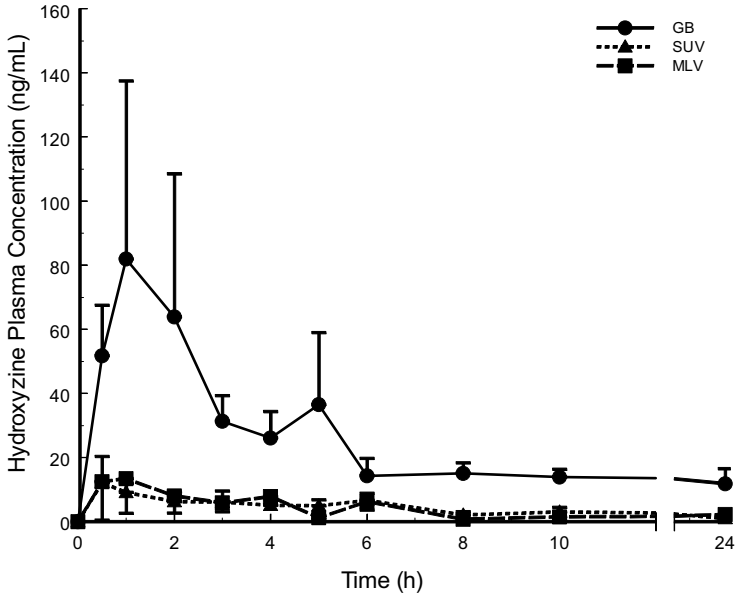

Figure 2. Mean (± SEM) hydroxyzine plasma concentrations after the topical application of 10 mg hydroxyzine from GB or SUV or MLV on the shaved backs of rabbits.

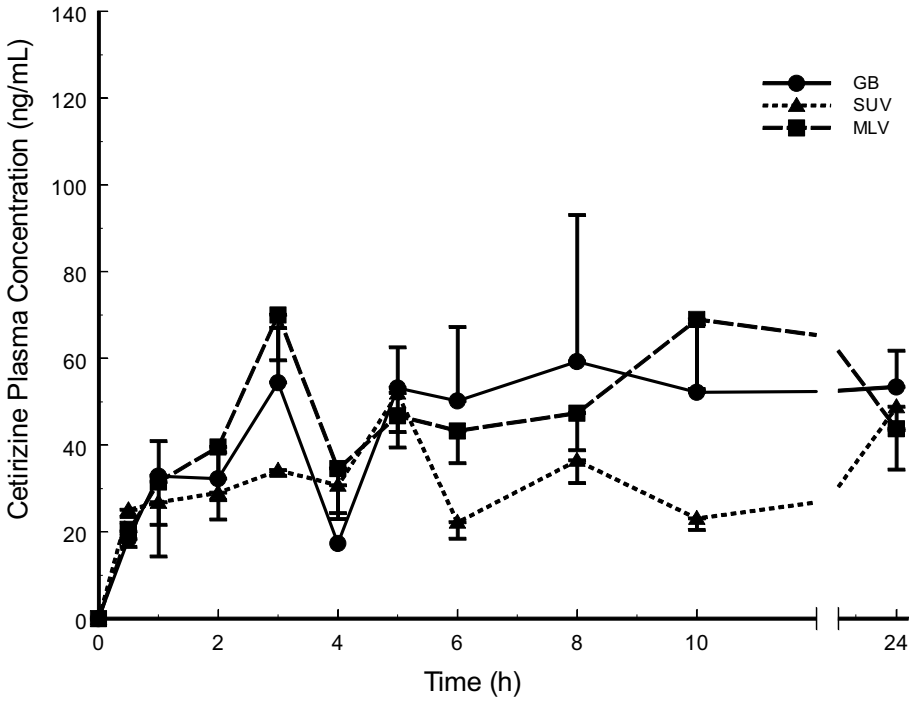

Figure 3. Mean (± SEM) cetirizine plasma concentrations after the topical application of 10 mg hydroxyzine from GB or SUV or MLV on the shaved backs of rabbits.

78.4 ± 33.9 ng.h/mL, respectively, over 24 hours, the mean plasma hydroxyzine concentrations from GB were significantly higher and ranged from 51.7 ± 15.8 to 11.9 ± 4.6 ng/mL, with an AUC of 492 ± 141 ng.h/mL ($P \leq .05$).

The extent of systemic absorption of hydroxyzine from these formulations was also evaluated by measuring the plasma concentrations of the hydroxyzine metabolite cetirizine as shown in **Figure 3**. There were no significant differences among mean plasma cetirizine concentrations ranging from 18.4 ± 3.7 to 70 ± 10.5 ng/mL arising from the hydroxyzine absorbed from PC-SUV (AUC of 765 ± 50 ng.h/mL), PC-MLV (AUC of 1035 ± 202 ng.h/mL), and GB (AUC of 957 ± 227 ng.h/mL) over 24 hours ($P \leq .05$).

At 24 hours after topical application of 10 mg hydroxyzine in PC-SUV, PC-MLV, and GB, the amount of the hydroxyzine dose remaining on the skin ranged from 0.02% ± 0.01% to 0.06% ± 0.02% of the applied dose, and there was no significant difference ($P \leq .05$) among the formulations.

DISCUSSION

Liposomes have been used as a carrier system to deliver medications into the skin in order to achieve the therapeutic effect with lower systemic absorption.[3] In both the SUV and MLV liposomes prepared for these studies, a high percentage of the amount of hydroxyzine incorporated into the formulations was entrapped within the liposome vesicles. The SUV and MLV liposome formulations, using phosphate buffer, pH 6.5, resulted in a mean entrapment of 86% ± 0.5% and 94.3% ± 0.4%, respectively. The extent of entrapment into the lipid vesicles was probably due to the lipophilicity of the neutral form of hydroxyzine in the liposome system (log P = 3.4), which was similar to that evaluated in an n-octanol/water system (log P = 3.5).[24]

We found that peripheral H_1-antihistaminic activity did not differ significantly after topical application of hydroxyzine in the 3 formulations tested. This finding may be attributed to the lipophilicity of hydroxyzine possibly leading to complete skin penetration of the drug regardless of formulation. Hydroxyzine from all formulations may penetrate to the dermo-epidermal junction, where the peripheral sensory nerves[25,26] are

found. The efficacy of H_1-antihistamines in skin disorders such as urticaria in humans is attributed primarily to their H_1-antihistaminic activity on small unmyelinated afferent C-fibers and reduction of itching.[27] In addition, H_1-antihistaminic activity on neurogenic reflexes reduces erythema/flaring, while H_1-antihistaminic activity on the endothelial cells of the postcapillary venules reduces leakage of the fluid and cells and resultant wheals.

The peripheral H_1-antihistaminic activity did not differ significantly among the formulations; however, lower plasma concentrations of hydroxyzine were found after application of PC-SUV and PC-MLV compared with GB. There was no significant difference between the plasma cetirizine concentrations measured after the application of PC-SUV, PC-MLV, or GB. Plasma cetirizine concentrations from all formulations were higher than hydroxyzine concentrations because cetirizine is hydrophilic and has a much smaller apparent volume of distribution.[28]

The hydroxyzine concentrations obtained after GB, when plotted versus time as shown in **Figure 2**, are similar to those that would be obtained after oral dosing, with a maximum concentration at 1 hour, followed by decreasing concentrations as the hydroxyzine was eliminated.[21] This result may be due to the hydroxyzine being released rapidly from the GB and absorbed quickly thorough the skin as a bolus dose. The lipophilic nature of the medication would permit rapid passage through the stratum corneum and the other dermal layers into the systemic circulation. Hydroxyzine has a relatively large volume of distribution, so it may redistribute into the skin layers and produce the potent peripheral antihistaminic effects seen in this study and shown previously in studies in human subjects.[21] The cetirizine concentrations achieved after the GB hydroxyzine administration would be consistent with rapid metabolism of hydroxyzine to cetirizine by the hepatic cytochrome P_{450} systems. The pattern of suppression of the histamine-induced wheals by hydroxyzine from GB, as shown in **Figure 1**, is consistent with the observed hydroxyzine plasma concentrations from GB as shown in **Figure 2** and from cetirizine as shown in **Figure 3**. An initial wheal suppression peak caused by the hydroxyzine is followed by a second wheal suppression peak caused by the formation of the active metabolite cetirizine.

The SUV and MLV liposome formulations yielded relatively low and persistent plasma hydroxyzine concentrations in conjunction with rapid onset and duration of significant peripheral H_1-antihistaminic effects, as monitored by suppression of the histamine-induced wheals. These results indicate that the liposomes may be causing the hydroxyzine to be concentrated in the skin. This could be achieved by the liposomes adsorbing to the skin surface intact, before penetrating through the stratum corneum. The smaller SUVs may penetrate the skin intact and then release the medication in a modified-release process during the ensuing 24 hours. The larger MLVs may shed the outer layers, slowly releasing some of the medication, and then penetrate the stratum corneum as oligolamellar vesicles, carrying the hydroxyzine into the dermo-epidermal junction and releasing the medication consistently over the 24 hours. This would explain the low concentrations of hydroxyzine in the systemic circulation from the 2 liposome formulations.

At the end of 24 hours, after the application of all 3 formulations, the amount of hydroxyzine remaining on the skin was very low compared with the initial dose applied. These results show that the low systemic concentrations of hydroxyzine following the application of the liposome formulations were not due to lack of absorption of the medication.

These results are in agreement with those of Mezei[26] and Foldvari et al[29] who have proposed several mechanisms to explain the liposome skin interactions and/or penetration. Multilamellar and unilamellar liposomes can be adsorbed to the skin surface intact before their penetration into the skin. Although larger liposomes may rupture on the skin surface releasing medication, smaller intact vesicles probably penetrate the skin. It is possible that intradermally localized unilamellar or oligolamellar vesicles are derived from multilamellar liposomes that have lost their outer bilayers by shedding during penetration. Foldvari et al[29] detected many intact SUV and to a lesser extent MLV liposomes, microscopically in pig skin, by using electron-dense colloidal iron-containing liposomes.

It is more difficult to account for the fact that plasma cetirizine concentrations arising from hydroxyzine after the SUV and MLV formulations were not significantly different from those obtained after GB. The smaller volume of distribution of cetirizine would partly account for the higher plasma oncentrations. If the hydroxyzine is concentrated in the skin from the SUV and MLV doses, then it is possible that it is metabolized to cetirizine in the skin, since the cytochrome P_{450} enzymes that metabolize it to cetirizine, are definitely present in the skin.[30] This requires further study. The cetirizine arising from the hydroxyzine from the SUV and MLV formulations likely also contributes to the suppression of the histamine-induced wheals.[22]

Although skin concentrations of hydroxyzine were not measured in our study, the low plasma hydroxyzine concentrations and the accompanying rapid and persistent suppression of the histamine-induced wheals are also in agreement with Foldvari et al.[29] These investigators found higher concentrations of ^{14}C-lidocaine in the epidermis and dermis of guinea pigs treated with liposome-encapsulated lidocaine compared with lidocaine in Dermabase (o/w) cream. Other investigators[31-34] found that the application of MLV liposomes prepared using phosphatidylcholine and loaded with hydrocortisone resulted in increased drug concentrations in the various layers of the skin epidermis and dermis, with an accompanying decrease in the serum concentrations, potentially leading to an increase in efficacy, while simultaneously decreasing the risk of adverse systemic effects. Foong et al[35] concluded that liposomal encapsulation of retinoids by soy phospatide and cholesterol can provide higher drug concentrations in the dermis and epidermis of albino guinea pig skin and lower drug concentrations in plasma and urine, in contrast to cream or gel dosage forms.

In summary, in this animal model we found that application of hydroxyzine in liposome formulations enhanced the peripheral H_1-antihistaminic effect of the drug and, at the same time, reduced systemic exposure to the drug. Further studies are required to determine the optimal liposomal formulation and to extend the findings of this study to humans with allergic skin disease.

ACKNOWLEDGEMENTS

This study was funded by an research grant from Pfizer, New York, NY. Also, we would like to acknowledge the Manitoba Health Research Council Graduate Studentship and University of Manitoba Graduate Fellowship for graduate student research scholarships awarded to Abeer Elzainy. In addition, we acknowledge the assistance of A. Halim Elamy, MSc, for the computer analysis.

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