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The release of clindamycin phosphate from a suspension of different types of liposomes and selected topical dosages forms

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Abstract

A Franz diffusion cells system was used to determine the release rate profile of clindamycin phosphate. Different types of liposomes containing clindamycin phosphate were prepared and the steady-state release of the drug, through a semipermeable synthetic membrane, was determined. The highest retention of clindamycin phosphate was seen from a suspension of multilamellar liposomes containing 1% clindamycin phosphate. Hydrogels, of acceptable consistency for topical use, were developed and used as carriers for a suspension of reverse-phase liposomes containing clindamycin phosphate. Increased penetration of clindamycin phosphate through a hairless mouse skin from a liposomes-containing vehicle compared with the permeation of the drug in a phosphate buffer was not observed.

Keywords: Clindamycin phosphate; Liposomes; Franz diffusion cell; Steady-state flux; Dermal drug delivery

1. Introduction

Acne is a very common ailment which most frequently develops during the teenage years. When choosing suitable therapy for mild to a moderate inflammatory acne, the topical use of antibiotics is in many cases the preferred method of treatment. Topical use of antibiotics is generally well tolerated compared with the systemic use which can cause considerable side effects. The antibiotics most commonly used for topical treatment of acne are clindamycin phosphate, erythromycin base, tetracycline hydrochloride and meclocycline sulfosalicylate (Arndt, 1991).

Several studies have compared topical with systemic antibiotic therapy. A double-blind study comparing topical use of clindamycin phosphate with oral use of minocycline in treatment of acne vulgaris reported that the efficacy of 1% topical clindamycin twice daily is comparable to oral use of minocycline 50 mg twice daily for patients with moderate to severe facial acne (Sheehan-Dare et al., 1990).

Clindamycin is generally considered the most effective topical antibiotic for acne (Rumsfield

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and West, 1992) but the main disadvantages of using clindamycin are the side effects, i.e. diarrhoea and the risk of pseudomembranous colitis, resulting from systemic absorption of the drug (Parry and Rha, 1986). A study of the absorption kinetics of topical clindamycin preparations has shown that percutaneous absorption of clindamycin is primarily dependent on the vehicle used (Eller et al., 1989).

The encapsulation of drugs into liposomes, for topical use, appears to give a higher drug concentration at the intended site of action, thereby enhancing the localized effects but at the same time minimizing unwanted systemic side effects (Mezei, 1985). Using liposomes as drug carriers for clindamycin phosphate would, therefore, have the possibility to decrease possible skin permeation of drug into the bloodstream, and also by enhancing the local effects on the skin, reduce the dosages regimen from twice daily to once a day and, therefore, have the possibility to increase patient compliance. Shalko et al. have reported improved clinical efficacy in the treatment of acne vulgaris when comparing liposomal clindamycin hydrochloride incorporated into multilamellar liposomes with non-liposomal drug (Skalko et al., 1992).

The aim of this study was to investigate the use of different types of liposomes as drug carriers for clindamycin phosphate. The objective was to ascertain which type of liposomes gave the highest release rate and to find a suitable dosages form (ointment base, carrier) to use as a vehicle for the liposomes. As the permeation of clindamycin phosphate depends on the vehicle used, the penetration of clindamycin phosphate, from a suspension of reverse-phase liposomes, was investigated through hairless mouse skin.

2. Materials and methods

2.1. Materials

Hydrogenated soya lecithin as a phospholipid (Phospholipon 90 H® (PL 90 H)) and Probiol® N 03017 cream (an emulsion that contains 6% phospholipid) were obtained as a gift from Nattermann Phospholipid GmbH, Cologne, Germany. Cholesterol (approx. 95% anhydrous) was purchased from Sigma Chemical Co., St. Louis, MO. Pro-lipo-H liposome concentrate was a gift from the Lucas Meyer Company, Hamburg, Germany. Clindamycin phosphate (CMP) was a gift from the Upjohn Comapny, Puurs, Belgium, and Carbopol® 940, was obtained from Nomeco, Copenhagen, Denmark. Dalacin® lotion, from the Upjohn Company, was used as a control. All other chemicals used were of special analytical grade. Synthetic semi-permeable cellophane membrane (Spectrapor® no. 2, molecular weight cut-off: 12 000–14 000) was obtained from spectrum Medical Industries, PA, U.S.A. Hairless mice (C3H/T if hr/hr) were obtained from Bommice, Denmark.

2.2. Methods

2.2.1. Preparation of liposomes

2.2.1.1. Preparation of reverse-phase liposomes. Reverse-phase evaporation vesicles were prepared according to Szoka and Papahajopoulos by the reverse-phase evaporation method (Szoka and Papahadjopoulos, 1978). Briefly, phospholipid (PL 90 H) and cholesterol in a 2:1 molar ratio were dissolved in an organic solvent, a mixture of dichloromethane and methanol (1:1). The aqueous phase, containing clindamycin phosphate (CMP) in a 0.05 M phosphate buffer, with pH 6, was then added to the solution. The solution was then sonicated in a bath-type sonicator (Kerry Ultrasonics Ltd.) at 20°C for 30 min. The resulting w/o emulsion was placed in a rotavapor connected with a vacuum pump (Millipore®). The organic phase was then removed, first at low vacuum (about 300 mm Hg), until foaming of the solution ceased, then the pressure was gradually increased until full vacuum was obtained. During this procedure the water bath was kept at 48°C. The final liposome preparation contained 1% CMP and 9.5 mg/ml (15 μmol/ml) lipid (Arnardóttir et al., 1995).

2.2.1.2. Preparation of multilamellar liposomes. Multilamellar liposomes were prepared by the

solvent evaporation method as originally described by Bangham (Bangham et al., 1974) and modified by Mezei (Mezei and Nugent, 1984). Briefly, Phospholipon 90 H and cholesterol in a 2:1 molar ratio were dissolved in an organic solvent (dichloromethane:methanol (1:1)) in a round bottom flask containing glass beads with diameter of 2.5 mm. The organic phase was then removed by a rotavapor, at 48°C, until the wall of the flask and the surface of the glass beads were covered with a thin lipid film. The film was then heated above the phase transition temperature of the phospholipid, up to 56°C, and then the aqueous phase, containing CMP in a 0.05 M phosphate buffer (pH 6) at 56°C, was added to the flask and handshaken vigorously for 1 min, in order to hydrate the lipid film. Finally, the flask was put into a thermostatically controlled shaker (Orbit enviromental shaker, LAB-LINE®) at 56°C and shaken, 160 rounds per min, for 15 min.

2.2.2. Preparation of liposomes from a commercial liposome concentrate

2.2.2.1. Preparation of Pro-lipo-H liposomes. Pro-lipo-H liposomes concentrate is like Probiol, a prepared liposome kit, consisting of hydrogenated soybean phosphatidylcholine (20%) and hydrophilic medium (80%). The concentrate appears as a cream at room temperature but converts to gel above 65°C. After dilution of the concentrate with distilled water (1:10), the liposomes obtained in this 2% suspension have the mean diameter of 300 nm and entrapped volume of 10 ml/g. The liposomes formed are oligo- and multilamellar vesicles. (All this information was obtained from the Lucas Meyer Company and was not confirmed experimentally by the authors).

Liposomes were produced, according to the manufacturers instructions, by heating up the liposome kit (65°C) and adding to it the aqueous phase (1:10) solution of CMP in a 0.05 M phosphate buffer (pH 6) at 65°C. After short agitation at 65°C the liposomes were prepared. The final liposome preparation contained 1% CMP.

2.2.3. Preparation of topical dosages forms

2.2.3.1. Preparation of hydrogels. Carbopol® 934 was added in small amounts to about 90% of the water phase used in the final product. While stirring, a 10% solution of methylparahydroxybenzoate in ethanol was added along with triethanolamine which was used to neutralise the prepared polymer solution (Remington's Pharmaceutical Sciences, 1990). After adding the rest of the water to the gel it was allowed to stand for 24 h, before mixing (2:3) with an appropriate amount of reverse-phase liposomes suspension containing 1% CMP. Finally, this mixture was centrifuged (Hermle Z 320, BHG) at 1000g for 5 min to improve the mixing of the liposomes suspension with the gel. For final composition of the gels, see Table 1.

2.2.3.2. Preparation of Probiol cream. Probiol® N 03017 cream is an emulsion consisting of 6% Phospholipon 90 H, 10% Mygliol® 812, 10% ethanol, 5% propylene glycol and the rest is water phase. Mygliol 812®, which is a trade name, consist of medium chain length triglycerides. These triglycerides are build up from fatty acids containing 8–10 carbon atoms (information obtained from the Nattermann Company). The

Table 1
The final composition of the liposome hydrogels

Material	Hydrogel A (%)	Hydrogel B (%)
Clindamycin phosphate	0.6	0.6
Carbopol 934®	0.8	2
Triethanolamine	1	2.5
Potassium dihydrogen phosphate	0.766	0.766
Ethanol	0.5	0.5
Phospholipon 90H	0.053	0.053
Methylparahydroxybenzoate	0.02	0.02
Cholesterol	0.013	0.013
Sodium hydroxide	0.0007	0.0007
Aqua destillata	ad 100	ad 100

emulsion was prepared according to the manufactures instructions. The aqueous solution of clindamycin phosphate in a 0.05 M phosphate buffer (pH 6) was mixed with a preweighted quantity of Probiol (1:3.5) until the cream became homogeneous. The obtained preparations contained 1% and 0.6% CMP, respectively.

2.2.4. In vitro drug release measurements

2.2.4.1. Diffusion cells and receptor fluid. Franz diffusion cells with 3,14 cm^2 surface area available for diffusion (type FDC-400 improved, Vangard International, Inc., Neptune, New Jersey) were used. The receptor phase was stirred constantly with a spinning bar magnet to ensure proper mixing (Shah et al., 1989). The receptor phase chosen for the measurements using a synthetic membrane was phosphate buffered saline (pH 6.4). The receptor phase chosen for the measurements using hairless mouse skin was the same, but included 0.4% (v/v) formaldehyde solution in order to prevent possible growth of microorganisms in the solution.

2.2.4.2. Determination of the steady-state flux of CMP through a semi-permeable synthetic membrane. Before application, the cellophane membrane was placed into saline solution for about 30 min in order to saturate it with the receptor phase. The membrane was then mounted between cell body and cap, and the cap was then clamped onto the cell body. The procedure was carried out at 37°C with thermostatically-controlled water circulating the external water jacket of the diffusion cell. About 2 ml, or 2 g, of each sample were added into the cap in order to cover the membrane; 100 μl samples were removed at predetermined time intervals, 40 min after the samples were placed on the membrane. After each sampling, 100 μl of fresh receptor fluid was replaced into the receptor chamber in order to keep the volume of the receptor phase constant. The obtained CMP concentrations were corrected according to the dilution of the receptor phase and the steady-state flux was determined from a plot of clindamycin released versus time (Shah et al., 1989).

2.2.4.3. In vitro skin permeation measurements. In order to determine the possible skin permeation of CMP, four female hairless mice were killed by cervical dislocation and their skin removed. The skin was first washed with 35% methanol following washing with water before it was put in the diffusion cell, with stratum corneum facing the donor chamber (Loftsson et al., 1994). A 2-ml sample of liposome suspension, reverse-phase liposomes, and 1% solution of CMP in a 0.05 M phosphate buffer (pH 6) as a control were added to the donor chamber. The procedure was carried out at 37°C, as previously described for the synthetic membrane, and 250-μl samples of the receptor phase were removed at predetermined intervals, the first sample 4 h after the specimen had been placed on the mouse skin. This study lasted 4 days.

2.2.5. Analytical procedure

Quantitative analysis of CMP was carried out by HPLC, using LKB 2150 pump (Pharmacia Biotec) and LKB 2138 uvicord wavelength monitor (Pharmacia Biotec) operated at 206 nm using Merck, LiChrospher® 100 RP-18 column (125*4 mm; 5μm). The mobile phase consisted, according to USP XXIII 1995, of 23% acetonitrile and 77% phosphate buffer (0.0775 M), adjusted to pH 2.5 with phosphorid acid, and the retention time was 3.0 min. The injection volume was 20μl and the flow rate was 1.5 ml/min.

3. Results

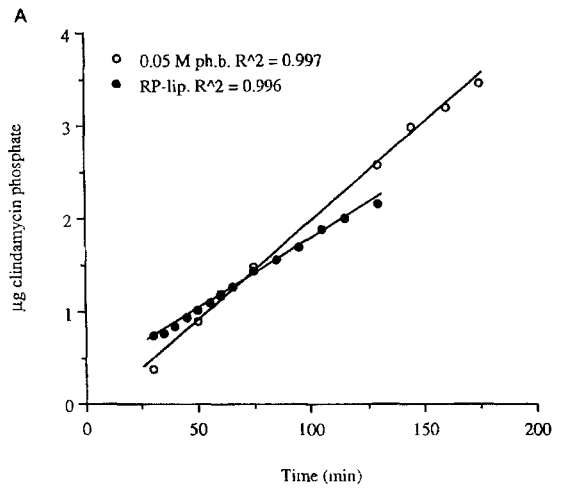
The mean (n = 3) cumulative flux of 1% clindamycin phosphate (CMP) through a semi-permeable synthetic membrane from three different liposome preparations (suspensions) were compared to the flux of CMP from a 0.05 M phosphate buffer solution containing the drug in the same concentration. The results from the diffusion experiments are shown in Fig. 1a, b and c. The results indicate that the flux is highest for the phosphate buffer where the drug exists entirely in uncapsulated form. The highest retention of CMP was obtained from a suspension of multilamellar liposomes containing 1% CMP (Fig. 1b).

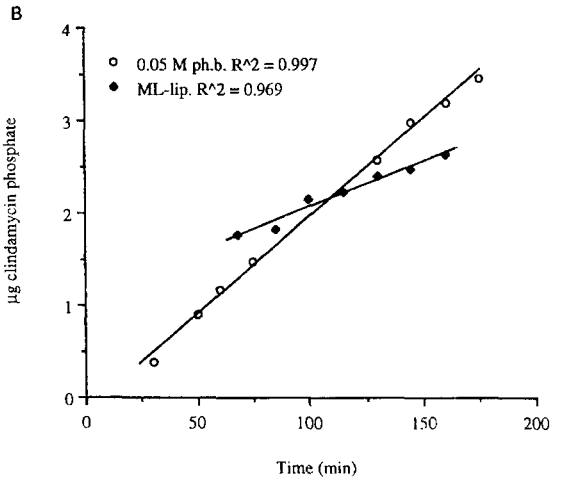
This flux can partly be explained by the flocculation tendency of suspensions of multilamellar liposomes. It is postulated that a layer of multilamellar liposomes could build up at the surface of the synthetic membrane in the donor phase of the diffusion cell, thereby partly blocking further passage of CMP through the membrane pores. Only marginal difference was detected for the flux of CMP from the phosphate buffer and the liposomes prepared from the commercial liposome concentrate Pro-lipo-H (Fig. 1c). This insignificant difference can perhaps be explained by less efficient loading of CMP into the Pro-lipo-H liposomes. Table 2 summarizes the steady-state flux values of the preparations shown in Fig. 1a, b and c.

In Fig. 2, the mean ($n = 3$) flux of CMP from the commercially available Dalacin lotion is compared with Probiol cream containing 1% CMP. Fig. 2 shows that there is only a small difference in the release rate of CMP from these two topical dosages forms.

Fig. 3 shows the mean ($n = 3$) flux of CMP from the liposome hydrogels A and B and the Probiol cream containing CMP in the same concentration (0.6%). Of the four different topical vehicles studied, the slowest release of CMP was from the Probiol cream. The results from the determination of the steady-state flux of CMP from the above mentioned topical dosages forms are shown in Table 3. Higher release of CMP from the Probiol cream containing 1% CMP, compared with the Probiol cream containing 0.6% CMP, can be explained by higher concentration of CMP in the 1% CMP containing vehicle.

The topical preparations summarized in Table 3 were all, apart from the commercial product Dalacin lotion, evaluated according to their appear-





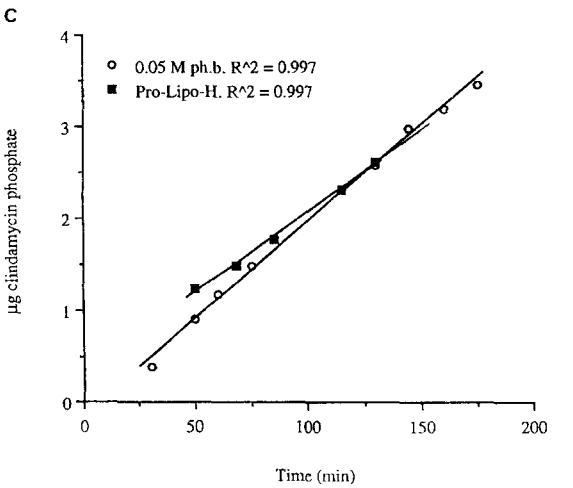


Fig. 1. (a) The mean ($n = 3$) steady-state flux of CMP (clindamycin phosphate) from a suspension of reverse-phase (RP) liposomes in comparison with the flux of CMP from a phosphate buffer (ph.b.) solution. Each vehicle contains 1% CMP. (b) The mean ($n = 3$) steady-state flux of CMP from a suspension of multilamellar liposomes (ML) in comparison with the flux of CMP from a phosphate buffer solution. Each vehicle contains 1% CMP. (c) The mean ($n = 3$) steady-state flux of CMP from a suspension of liposomes produced from Pro-lipo-H® liposome concentrate in comparison with the flux of CMP from a phosphate buffer solution. Each vehicle contains 1% CMP.

Table 2
The steady-state flux of 1% solution of CMP from three different types of liposome suspensions

Vehicle	Mean steady-state flux ± S.D. ($\mu g/cm^2$ per min)
0.05 M phosphate buffer	6.93 ± 0.80
RP-liposomes[a]	4.73 ± 0.14
ML-liposomes[b]	3.42 ± 0.41
Pro-Lipo-H®-liposomes	5.25 ± 0.36

[a]RP, Reverse-phase liposomes.
[b]ML, Multilamellar liposomes.

ance, feel and visual stability (Hanna, 1989). The hydrogels A and B appeared as milky white hydrogels. Hydrogel A appeared to be more aqueous but less sticky than hydrogel B which contains greater amount of the gel forming agent. Hydrogel B, when applied on the skin, had shorter drying time than hydrogel A, but hydrogel A was easier to apply. Probiol cream, containing CMP in a 0.6% and 1% concentration, appeared as a white, homogeneous cream. It spreads very easily over the skin surface but had a longer drying time than the hydrogels. Unlike the hydrogels, it left the skin surface with the sence of increased hydration, most likely due to the higher content of other fat components than in the liposome hydrogels.

Study of possible skin penetration of CMP through a mouse skin indicates poor ability of clindamycin phosphate to penetrate the skin. Smaller than 0.00004% of CMP, from a suspension of reverse phase liposomes containing 1% CMP and the control, 1% CMP in a 0.05 M phosphate buffer, penetrated in 4 days through the mouse skin.

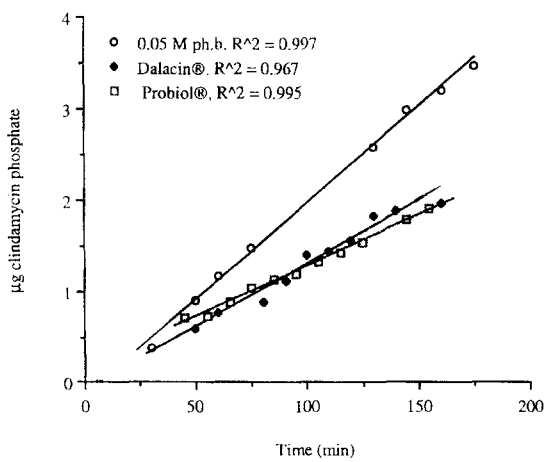


Fig. 2. The mean (n = 3) steady-state flux of Probiol cream containing 1% CMP and Dalacin® lotion in comparison with the flux of CMP from a phosphate buffer solution.

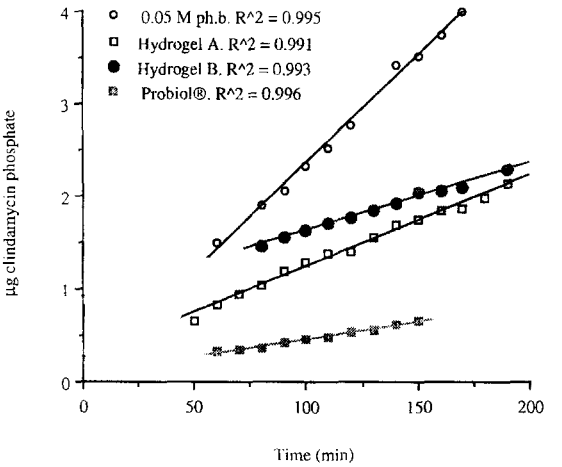


Fig. 3. The mean (n = 3) steady-state flux of CMP from Probiol cream, Hydrogel A and B in comparison with the flux of CMP from a phosphate buffer solution. Each vehicle contains 0.6% CMP.

4. Conclusion

Comparison of the release rate profiles of CMP from different types of liposomes suspension through a semi-permeable synthetic membrane showed highest release rate of CMP from the commercially availuable liposome concentrate Prolipo-H. The greatest retention of CMP release was observed from a suspension of multilamellar liposomes containing 1% CMP.

Increased permeation of the drug through hairless mouse skin, from a suspension of liposomes containing CMP compaired with the drug in a phosphate buffer, was not observed.

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Table 3
The mean steady-state flux of the selected topical liposomes-dosages forms studied

Vehicle	% Clindamycin phosphate	Mean steady-state flux ± S.D ($\mu g/cm^2$ per min)
RP-liposomes in hydrogel A	0.6	3.26 ± 0.16
RP-liposomes in hydrogel B	0.6	2.36 ± 0.38
Probiol® cream	0.6	1.25 ± 0.30
Probiol® cream	1	3.33 ± 0.18

damycin phosphate, the Nattermann Phospholipid GmbH, Cologne, Germany, for donating the sample of Probiol® and the Lucas Meyer Company, Hamburg, Germany, for donating the sample of Pro-lipo-H® liposomes.

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