International Journal of Pharmaceutics, 85 (1992) 97–101

IJP 02779

Liposomes with clindamycin hydrochloride in the therapy of Acne vulgaris

Nataša Škalko, Mira Čajkovac and Ivan Jalšenjak

Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb (Croatia)

(Received 14 May 1991)
(Modified version received 23 October 1991)
(Accepted 30 January 1992)

Key words: Liposome; Clindamycin hydrochloride; Acne vulgaris

Summary

Liposomes with clindamycin hydrochloride were prepared using either soya lecithin and cholesterol or hostaphate and cholesterol. In vitro dissolution studies showed sustained release of the drug from hostaphate liposomes compared to lecithin liposomes. Clinical treatment of Acne vulgaris with a lotion of liposomal drug shows better efficacy than non-liposome lotion forms (especially of the treatment of pustules where clinical improvement was 77% of initial number). Application of a conventional lotion solution, a non-liposomal emulsion lotion and a liposomal emulsion lotion resulted in decreases of 42.9, 48.3 and 62.8%, respectively, in the total number of lesions after a 4 week treatment. The result support the possibility of developing products utilizing the liposomal dosage form that are superior to existing dosage forms for topical therapy.

Introduction

Liposomes, minute spherical vesicles consisting of lipid bilayers, have been suggested for use as a suitable 'carrier' or 'localizer' for various drugs used in topical administration. According to Egbaria and Weiner (1990), liposomal drug formulations are able to reduce side effects and incompatibilities, to enhance the accumulation of drug at the administration site and to incorporate a variety of hydrophilic and hydrophobic drugs.

Correspondence to: I. Jalšenjak, Faculty of Pharmacy and Biochemistry, University of Zagreb, 41000 Zagreb, A. Kovačića 1, Croatia.

Recently, several reports have been published describing results for various liposomal drug formulations, the drugs being as follows: triamcinolone acetonide (Mezei and Gulasekharam, 1980, 1982; Krowczynski and Stozek, 1984), dihydrotestosterone (Vermorken et al., 1984), progesterone and hydrocortisone (Ganesan et al., 1984), progesterone (Rowe et al., 1984; Mezei, 1985), methotrexate (Patel, 1985), butylparaben (Komatsu et al., 1986a,b), glucocorticoids (Lasch and Wohlrab, 1986; Wohlrab and Lasch, 1987, 1989; Wohlrab et al., 1989), and diclofenac (Nishihata et al., 1987). More recently, two excellent reviews (Mezei, 1988; Egbaria and Weiner, 1990) have appeared dealing with the various aspects of liposomes used as topical drug delivery systems.

It should be noted that although liposomes are promising for potential use in pharmacotherapy, clinical studies are still scarce. Therefore, we have attempted to study a liposomal clindamycin hydrochloride formulation in comparison to a standard, well established preparation used in hospitals against one of the most common skin diseases (Acne vulgaris).

Materials and Methods

Materials

Clindamycin hydrochloride (according to USP XXII), soya lecithin (BDH, UK), Hostaphat KW 340 N and Hostacerin T 3 (Hoechst, Germany) were used in all experiments as received. All other chemicals were of reagent grade of pharmacopoeal purity.

Preparation of liposomes

Liposomes were prepared essentially according to the same technique that was first reported by Bangham et al. (1965). 10 ml of lecithin in chloroform (10 mg/ml) and 2 ml of cholesterol in chloroform (5 mg/ml) or hostaphate and cholesterol (at the same ratio) were subjected to treatment in a rotary evaporator at 40°C until the solvent had been completely removed. A solution of clindamycin hydrochloride (20 ml; 1 or 5% w/v) was added to a flask and MLV liposomes were formed during mild agitation. Size analysis of the resulting liposomes was performed on an image analysis system (Optomax, AMS, U.K.). A dye specific for lipids (Sudan Rot R; Schering, Germany) was used to achieve better contrast.

In vitro test of antibacterial activity

Dialysis of the drug from a liposome suspension sample (5 ml) into water (95 ml) through a Cuprophan membrane (Gambro, Sweden) was measured. At predetermined time intervals, samples (1 μl) were taken and the drug was determined by a microbiological method.

The method of agar diffusion was used in detecting the antibacterial activity of clindamycin hydrochloride against *Sarcina lutea* 9341 ATCC (Brown and Beyer, 1981). Sterile molten Muller-Hinton medium maintained at 45°C was inoculated with 10^6 cells of the microorganism and poured into sterile Petri dishes to form a layer 4 mm thick. Samples of drug solution obtained by dialysis were placed on agar plates with a micropippete. After 24 h of incubation at 37°C, the zones of inhibition were measured. Clindamycin hydrochloride concentrations were determined vs a standard curve previously prepared. Each value represented an average value of six measurements.

Preparation of dosage forms

In the clinical study three lotion preparations were made up as follows. (I) Standard clindamycin hydrochloride lotion: the drug was dissolved in a mixture of propylene glycol (10% v/v) and ethyl alcohol (70% v/v) (Algra et al., 1977); (II) free clindamycin hydrochloride lotion-emulsion was prepared from Hostaphat KW 340 N (2% w/w), Hostacerin T 3 (2% w/w), liquid paraffin (8% w/w), distilled water (88% w/w) and preserving agent. Drug solution (5% w/v) was added to the emulsion previously prepared of ratio 4:1; (III) liposomal clindamycin hydrochloride lotion-liposome suspension (5% w/v) was added to the emulsion (made up as in lotion II) of ratio 4:1. The final drug concentration was 1% in all lotions prepared.

Clinical studies

Two sets of trials were performed in order to compare the non-liposomal preparations with the liposomal type (lotion III), namely, for study A (with lotion I) and study B (with lotion II). Prior to clinical trials approval was obtained from the hospital ethical commission and the consent of the patients. All skin lesions were located on the facial area. The patients had been undergoing treatment with different drugs prior to entering the study and had been suffering from skin complaints for 3 months to 12 years before the period of study. Previously treatments were discontinued at least 30 days prior to these studies. During the investigation, tests for irritancy and sensitivity were performed but no positive reactions were observed.

Study A 30 volunteers aged 18.8 ± 0.4 years (23 female and 7 male) participated in the study.

Regarding skin type, five patients had oily skin and 25 medium oily skin. Treatment was continued for 4 weeks. Patients were instructed to apply the lotions with cotton balls to the affected areas twice daily (morning and night). Lotion III was applied on the right side of the face and lotion I on the left. During the study, the number of skin lesions was always counted on the same face area (4 cm^2) marked with a paper template.

Study B The study involved 30 patients with acne (21 female and 9 male; aged 19.5 ± 0.5 years). 10 patients had extremely oily skin, 18 had oily skin and two had normal skin. Patients applied lotions exactly in the same way as in study A, the only difference being the replacement of lotion I by lotion II.

Results and Discussion

The method of liposome preparation used in this study resulted in the reproducible production of samples of MLV liposomes. The main characteristics of liposomes thus prepared are given in Table 1. The average diameters of liposomes were basically the same among all preparations, although a definite shift toward the higher end of the range of values can be observed for samples having 5% of drug. The drug release from liposomes was influenced by the drug content and the type of preparation. The 5% samples released their drug content more slowly than those of 1% and hostaphate liposomes sustained drug release longer than lecithin liposomes. This phenomenon

TABLE 1

Characteristics of clindamycin hydrochloride liposome samples [a]

Type of liposomes	Drug content (%)	Average diameter size (μm)	Range of sizes (μm)	$t_{50\%}$ in vitro (days) [e]	$t_{100\%}$ in vitro (days) [e]
L [b]	1	1.7	0.77–6.78 [d]	0.1	2
L	5	2.0	0.77–8.47	0.2	3
H [c]	1	2.1	0.77–6.78	0.8	3
H	5	1.9	0.77–8.47	1.4	4

[a] Mean of two evaluations.
[b] Liposomes made from lecithin.
[c] Liposomes made from hostaphate.
[d] Lower limit of detection.
[e] Time needed for 50% or 100% release of the drug from liposomes as measured by a dialysis through a Cuprophan membrane.

TABLE 2

Decrease (in percentage) of separate and total [a] *number of skin lesions after 2 and 4 weeks of treatment*

Lotion (study)	Time (weeks)	Comedones	Papules	Pustules	Total
I (study A)	2	25	32	43	34
	4	31	39	46	43
II (study B)	2	15	17	32	24
	4	38	50	62	49
III	2	30	51	58	46
	4	44	66	76	63

[a] Total initial number of lesions was approx. 400.

TABLE 3

Final evaluation indices after 4 weeks treatment [a,b,c]

Lotion (study)	Comedones	Papules	Pustules
I (study A)	1.60	1.60	1.10
II (study B)	1.50	1.10	0.70
III	1.05	0.45	0.35

[a] Initial index in all cases was approx. 3.
[b] 0, excellent (> 66% improvement); 1, very good (33–66%); 2, good (< 33% improvement); 3, no changes.
[c] The sum of all individual patients' indices was calculated and divided by the number of patients.

was not investigated further. The hostaphate liposomes were later included in the clinical trials, since Patel (1985) has suggested that liposomal preparations may act as vehicles for the sustained release of drugs into the skin. Obviously then, liposomes with a longer duration of in vitro drug release should be preferable as drug delivery systems for in vivo testing.

The results of clinical studies were classified on two levels as follows. Table 2 lists the clinical improvement expressed as the decrease in skin lesions after the 2 or 4 week treatment and was counted by an observer. The total number of lesions consisted of different manifestations of Acne vulgaris (e.g., comedones, papules and pustules). It is evident that all manifestations of the disease were not treated equally well. On a separate level, the final skin condition of patients was evaluated after a 4 week treatment by an independent clinical physician, the results being summarized in Table 3 as evaluation indices.

It is evident that the treatment with a liposomal form of clindamycin hydrochloride in both studies produced better results both for the decrease in total number (in percentage) of skin lesions (Table 2) and for the physician's evaluation of skin status before and after treatment (Table 3).

Considering the duration of treatment, it should be noted that lotion I achieved most of its activity during the first 2 weeks of treatment while with lotions II and III further improvement was gained by prolonging the application of preparations up to 4 weeks. After that time, no noticeable improvement was observed. It is evident that even lotion II gave better results than the standard preparation (lotion I), however, liposomal entrapment (lotion III) of the drug was crucial for attaining the best results.

In conclusion, the clindamycin hydrochloride liposomes have real potential for use in the treatment of Acne vulgaris. However, the question concerning the mechanism of liposome at action on the skin remains to be resolved separately. Also, further work will show whether the observed efficacy of clindamycin hydrochloride liposomes can be improved even more by using other types of liposomes.

References

Algra, R.J., Rosen, T. and Waisman, M., Topical clindamycin in Acne vulgaris. *Arch. Dermatol.*, 111 (1977) 1390–1391.

Bangham, A.D., Standish, M.M. and Watkins, J.C., Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.*, 13 (1965) 238–252.

Brown, L.W. and Beyer, W.F., Clindamycin hydrochloride. In Florey, K. (Ed.), *Analytical Profiles of Drug Substances,* Academic Press, New York, 1981, pp. 75–91.

Egbaria, K. and Weiner, N., Liposomes as a topical drug delivery system. *Adv. Drug. Del. Rev.*, 5 (1990) 287–300.

Ganesan, M.G., Weiner, N.D., Flynn, G.L. and Ho, N.F.H., Influence of liposomal drug entrapment on percutaneous absorption. *Int. J. Pharm.*, 20 (1984) 139–154.

Komatsu, H., Higaki, K., Okamoto, H., Miyagawa, K., Hashida, M. and Sezaki, H., Preservative activity and in-vivo percutaneous penetration of butylparaben entrapped in liposomes. *Chem. Pharm. Bull.*, 34 (1986a) 3415–3422.

Komatsu, H., Okamoto, H., Miyagawa, K., Hashida, M. and Sezaki, H., Percutaneous absorption of butylparaben from liposomes. *Chem. Pharm. Bull.*, 34 (1986b) 3423–3430.

Krowczynski, K. and Stozek, T., Liposome als Wirkstoffträger in der Percutanen Therapie. *Pharmazie,* 39 (1984) 627–629.

Lasch, J. and Wohlrab, W., Liposome-bound cortisol: A new approach to cutaneous therapy. *Biomed. Biochim. Acta,* 45 (1986) 1295–1299.

Mezei, M. and Gulasekharam, V., Liposomes – A selective drug delivery system for the topical route of administration. Lotion dosage form. *Life Sci.*, 26 (1980) 1473–1477.

Mezei, M. and Gulasekharam, V., Liposomes – A selective drug delivery system for the topical route of administration: Gel dosage form. *J. Pharm. Pharmacol.*, 34 (1982) 473–474.

Mezei, M., Liposomes as a skin drug delivery system. In Breimer, D.D. and Speiser, P. (Eds), *Topics in Pharmaceutical Sciences,* Elsevier, Amsterdam, 1985, pp. 345–358.

Mezei, M., Liposomes in the topical application of drugs: a review. In Gregoriadis, G. (Ed.), *Liposomes as Drug Carriers, Recent Trends and Progress*, Wiley, Chichester, 1988, pp. 663–677.

Nishihata, T., Kotera, K., Nakano, Y. and Yamazaki, M., Rat percutaneous transport of diclofenac and influence of hydrogenated soya phospholipids. *Chem. Pharm. Bull.*, 35 (1987) 3807–3812.

Patel, H.M., Liposomes as a controlled-release system. *Biochem. Soc. Trans.*, 13 (1985) 513–516.

Rowe, T.C., Mezei, M. and Hilchie, J., Treatment of hirsutism with liposomal progesterone. *Prostate*, 5 (1984) 346–347.

Vermorken, A.J.M., Hukkelhoven, M.W.A.C., Vermeesch-Markstag, A.M.G., Goos, C.M.A.A., Wirtz, P. and Ziegenmeyer, J., The use of liposomes in the topical application of steroids, *J. Pharm. Pharmacol.*, 36 (1984) 334–336.

Wohlrab, W., Lackmann, U. and Lasch, J., Penetration of lecitin from hydrocortisone-containing liposomes into human skin. *Dermatol. Mon. Schr.*, 175 (1989) 344–347.

Wohlrab, W. and Lasch, J., Penetration kinetics of liposomal hydrocortisone in human skin, *Dermatologica*, 174 (1987) 18–22.

Wohlrab, W. and Lasch, J., The effect of liposomal incorporation of topically applied hydrocortisone on its serum concentration and urinary excretion. *Dermatol. Mon. Schr.*, 175 (1989) 348–352.