Pharmacy Department[1], Faculty of Technology and Engineering, M.S. University of Baroda, and S.S.G. Hospital[2], Government Medical College, Kothi, Baroda, India

A topical dosage form of liposomal clofazimine: research and clinical implications

V. B. Patel[1], A. N. Misra[1] and Y. S. Marfatia[2]

A novel topical clofazimine (CLO) gel formulation containing liposomally encapsulated CLO, was prepared and investigated *in vitro* followed by a clinical evaluation. CLO liposomes were prepared by the lipid film hydration technique. Comparative *in vitro* diffusion studies were conducted with plain and liposomal CLO in HPMC K4M gel base (2% and 5%) using human cadaver skin (HCS). A double blind clinical study was conducted on eight leprosy patients. The results of these studies show that the new liposomal topical gel formulation not only prolongs the drug release but also promotes drug retention by the skin. Studies further support formation of a reservoir of drug on the skin modifying therapeutic efficacy of the formulation. The new liposomal gel formulation of CLO considerably reduces the healing time of external lesions due to a significantly prolonged skin residence time compared to plain CLO gel and hence is expected to reduce the time needed for leprosy treatment.

1. Introduction

Leprosy affects mostly face, eyes, nose and lymph glands. The only oral therapy, with an average duration of 2–5 years, suffers from the drawback of major side effects due to long term administration of clofazimine (CLO). Liposomal topical formulations have shown a great potential as drug delivery systems [1–6]. Hence, an attempt was made to formulate a supplement dermal therapy of CLO. The liposomal encapsulation of CLO was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy.

CLO liposomes were prepared by a reported lipid film hydration technique after optimizing process and formulation variables.

The present investigation focusses on comparative *in vitro* release studies and the clinical evaluation of new liposomal gel formulations on indoor leprosy patients.

2. Investigations, results and discussion

The method of liposome preparation used in this study [7] resulted in a reproducible production of MLV liposomes.

The *in vitro* permeation of CLO through human cadaver skin (HCS) from the novel liposomal gel formulations, CLO6 and CLO7 was evaluated and compared with that of a plain drug gel formulation, P, all containing 3% w/w CLO, using a self-designed and validated diffusion cell. Acetate buffer pH 5.0 with 20% PEG 400 was used as diffusion medium. In the validation of the *in vitro* cell, the calculated F value (3.01) was less than the table F value (3.74, $f1 = 5 : f2 = 24$) at $P < 0.01$ indicating that all the six samples withdrawn from the diffusion cell did not vary significantly from each other. In a single study, 0.5 g of gel formulation was applied to the donor compartment and the study was continued up to 72 h. At each sampling point, the whole fluid of the receptor compartment was withdrawn and replaced with fresh fluid. The withdrawn fluid was analysed for the amount of drug diffused in the period. The mean cumulative amount of CLO diffused (Q, $\mu g/cm^2$) at each sampling point was calculated and the data obtained (Table 1) were subjected to the regression analysis. Fig. 1 shows the plot of regressed Q vs. $t^{1/2}$. From the graph, it is evident that significantly ($P < 0.05$) more CLO diffused from P than from CLO6 and CLO7. The results indicate that liposomal encapsulation of CLO

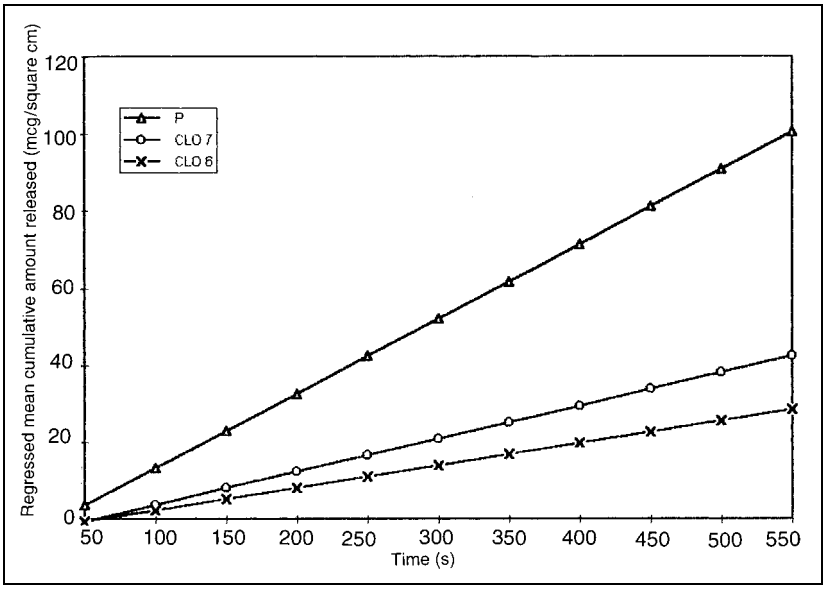

Fig. 1:
Diffusion studies of P, CLO6 and CLO7

Table 1: Diffusion studies of P, CLO6 and CLO7

Time (h)	Time ($s^{1/2}$)	Mean cumulative amount released* (μg/cm²)		
		P	CLO6	CLO7
1	60	2.50	0.80	1.02
2	34.85	5.57	1.87	2.92
4	120	18.10	3.38	4.90
6	146.97	30.67	5.75	7.64
24	293.94	51.24	11.19	19.00
48	415.69	72.27	19.24	29.24
72	509.12	92.50	28.07	41.00

* = Mean of six determinations

potentially prolongs the drug release across HCS [8–10]. An attempt was made to understand the diffusion pattern. When the data of mean cumulative drug released vs. $t^{1/2}$ was plotted and subjected to regression analysis, a correlation coefficient r between 0.992–0.996 was found for the liposomal formulations. For the plain drug gel formulation r was found to be 0.992 which indicates that a linear relationship exists between these two parameters and proves that the release obeys the Higuchi's diffusion controlled model [11]. The slope of the line represents the release rate [12]. The data suggest a diffusion of drug from the liposomes to the gel base and then across the HCS and this indicates a substantial reduction in the mean flux values compared to the plain CLO gel formulation. The mean flux values (Fig. 2) of P were also found to be 3.76 times and 3.06 times higher compared to the novel liposomal gel formulations, CLO6 and CLO7, respectively. This further confirms the reduction in diffusion rate of the drug across HCS after liposomal encapsulation.

When compared amongst the liposomal batches, no statistically significant difference was observed in the diffusion pattern up to 6 h after which CLO7 was found to release more drug than CLO6 (Table 1). This may be due to a higher amount of cholesterol in CLO6 retarding the release by reducing the permeability of the liposomal membrane [13]. This agrees with the findings of Demel et al. [14].

Liposomal encapsulation of a drug was found to enhance the drug biodisposition in the skin [8, 15, 16]. In this investigation, the *in vitro* deposition of CLO in human skin, was compared between P containing the free drug and CLO6 and CLO7 containing liposomally encapsulated drug. The amounts of CLO localized in human skin, after a 72 h exposure period and removing off more than 99% of the formulation [17], are listed in Table 2. As expected, the novel gel formulations, CLO6 and CLO7 showed significantly greater deposition of CLO in the skin than P at $P < 0.05$.

All the above studies carried out with 2% HPMC K4M gel base were also repeated with a 5% HPMC K4M gel base. The results of a 72 h study with the 5% gel base (Fig. 2) indicated a dramatic decrease in the mean flux values which may be correlated with the sustained release pattern due to the higher viscosity of the gel base. At the same time, the skin deposition of the drug was significantly reduced (Table 2). Hence, 2% gel formulations were selected for the clinical evaluations.

Clinical evaluations were carried out based on the healing time of external lesions and skin staining due to CLO. The results of the clinical studies were classified on two levels [18] as follows. Fig. 3 shows the five point scale wherein the clinical improvement is expressed as percent reduction in dimension of skin lesions after 12 weeks treatment (measured using vernier calipers by the physician in charge). On a separate level, the final skin condition of patients was evaluated after 12 weeks treatment by an independent group of clinical physicians, the results being summarised in Table 3. It is evident that the novel liposomal CLO gel formulations, CLO6 and CLO7, de-

Table 2: *In vitro* deposition of CLO in human skin from 3% CLO gel following mild washing of the skin surface with diffusion medium

Test formulation	Human skin (%) mean (SEM)		Washing medium (%) mean (SEM)	
	A*	B*	A*	B*
P	32.45 (0.115)	21.46 (0.131)	51.73 (0.147)	66.92 (0.087)
CLO6	65.03 (0.256)	52.27 (0.140)	28.60 (0.177)	44.00 (0.112)
CLO7	57.18 (0.109)	47.00 (0.156)	34.04 (0.108)	47.88 (0.240)

*A = 2% gel base and B = 5% gel base

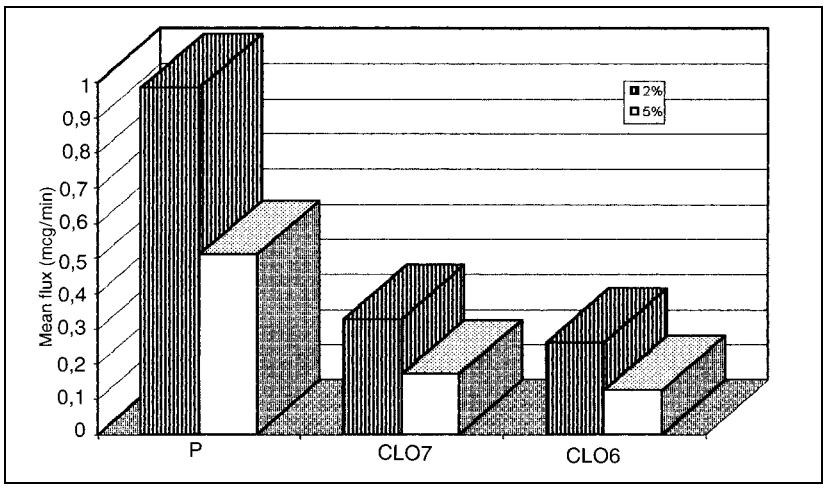

Fig. 2:
Mean flux values of P, CLO7 and CLO6 from 2% and 5% HPMC K4M gel bases

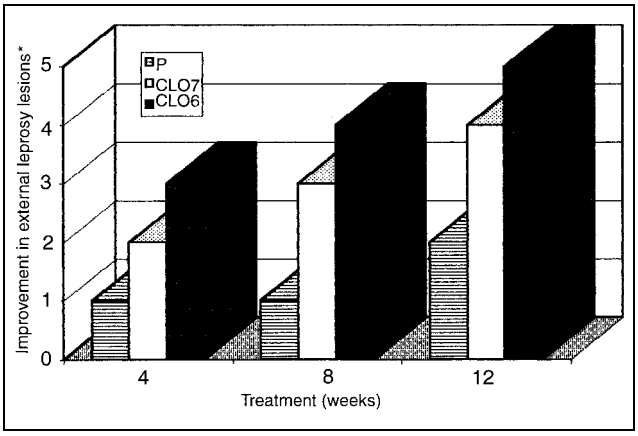

Fig. 3: Clinical evaluations in terms of reduction in dimension of external leprosy lesions
* Improvement: 1: 0–10%, 2: 10–20%, 3: 20–40%, 4: 40–60%, 5: 60–80%

Table 3: Skin examination for staining due to CLO

Test formulation	Skin condition*	
	Before treatment	After treatment
P	2.0	1.0
CLO6	2.0	4.0
CLO7	2.0	4.0

* Reddening 1: Severe, 2: Moderate, 3: Low, 4: Nonexistent

Table 4: Analytical profile of liposomal batches*

Batch	PC : CHOL Molar ratio	CLO : PC : CHOL Molar ratio	Encapsulation efficiency (%) mean ± S.D.
CLO6	5.13 : 1.00	1 : 5.00 : 1.00	61.02 ± 0.33
CLO7	7.70 : 1.00	1 : 7.85 : 1.02	71.00 ± 0.053

* Average of six determinations

creased the healing time of external skin lesions considerably as compared to the plain drug gel formulation, P. Moreover, the skin staining due to CLO was considerably reduced by liposomal encapsulation. The liposomal formulations produced better results at both levels (Fig. 3 at $P < 0.01$ and Table 3 at $P < 0.01$).

Several *in vitro* percutaneous absorption studies employing 3% w/w CLO as a model compound have shown that a novel gel formulation containing CLO in a liposomal form significantly reduces the transdermal drug absorption while potentially enhancing both the skin deposition and residence time of CLO compared with a plain CLO gel formulation. The results of these studies support a mechanism of formation of a CLO reservoir on the skin surface [8]. Hence, it may be concluded that the liposomal CLO gel formulation may have a role in supplementing oral therapy in leprosy patients reducing the time period of therapy substantially. It is also expected to reduce the side effects. However, the role of the formulations of this investigation may only be settled after clinical evaluation with a larger number of patients.

3. Experimental

3.1. Materials

Clofazimine I.P. (CLO, Gift sample by Asoj Soft Caps Pvt. Ltd., India). Egg phosphatidyl choline (PC) (Centre for Biochemical Technology, Delhi, India), cholesterol A.R. (CHOL) (S.D. Fine Chem. Ltd., India), α-tocopherol (α-toco) (E. Merck), HPMC K4M (Dow Chemical Company, U.S.), sephadex (Pharmacia LKB, Sweden), polyethylene glycol 400 (PEG 400) (E. Merck) were purchased and used as such without further purification. All other chemicals used were of analytical grade.

Acetate buffer (pH 5.0, ionic strength 0.261) was prepared as described in the Indian Pharmacopoeia 1996 [19].

3.2. Preparation of skin

Human cadaver skin (HCS) was obtained from autopsy at the Faculty of Medicine, M.S. Univ. of Baroda. It was treated suitably and stored at −4 °C. Full thickness HCS membrane was prepared by shaving the skin, punching out a tissue of approximately 5.0 cm^2 area, trimming away the excess fat and slicing to 500 μm thickness using a Davis Dermatome 7. These sclices were hydrated with diffusion medium (pH 5.0 acetate buffer with 20% PEG 400) 24 h prior to use.

3.3. Preparation of the liposomes

Films of CLO, PC, CHOL in the ratio shown in Table 4 and α-toco (1% w/w of PC taken) were prepared in 100 ml round bottom quickfit flasks by rotary evaporation under vacuum at 25 ± 2 °C (180 rpm, the speed of rotation of the flask) of a chloroform/methanol (2 : 1) solution system. The film was hydrated with pH 5.0 acetate buffer for 2 h, sonicated for size reduction for 6, 2 min intervals using an icebath and hydrated for 24 h [7].

3.4. Preparation of the liposomal gels

Gel base (2% w/w and 5% w/w) was prepared by separately dispersing 20 g and 50 g of HPMC K4M, respectively in 980 ml/950 ml of distilled water, containing 0.001% w/v of phenyl mercuric nitrate, using a triple blade stirrer. The liposomal suspensions were centrifuged and the pellets obtained were incorporated into each of the prepared gel bases to get 3% w/w of CLO in the gel base.

In the clinical study, three gel formulations were made as follows: Plain CLO gel (P) was prepared by triturating CLO with HPMC K4M gel base. Liposomal CLO gels CLO6 and CLO7 were prepared by incorporating CLO6 and CLO7, respectively into HPMC K4M gel base by trituration. The final CLO concentration was 3% w/w in all the gels prepared. The gels were filled into 20 g lacquer coated aluminium tubes and sealed securely. The tubes were coded by a person not involved in the study.

3.5. Diffusion studies

A modified diffusion test apparatus [20–22] with a diffusional area of 19.643 cm^2 was used for the permeation studies and was validated by the benzoic acid disc method [23]. Each study was conducted using dermatomed and prepared HCS from the same donor (500 μm thickness). 0.5 g of formulation (Plain CLO formulation P and liposomal formulations CLO6 and CLO7) was applied to the skin (hydrated in the diffusion medium 24 h prior to the study), tied over the funnel with plastic film (parafilm) and immersed in 50 ml diffusion medium (pH 5.0 buffer with 20% PEG 400 as a cosolvent) maintained at 37 ± 0.5 °C under continuous stirring at a rate of 50 rpm in a way that the dermal surface just flushes to the surface of the diffusion fluid.

Serial sampling of the dermal compartment was performed at specified times (1, 2, 4, 6, 24, 48 and 72 h) by totally removing the contents of the receptor and refilling with fresh medium. The amount of CLO diffused was analysed on a U-2000 UV-Visible Spectrophotometer at 535 nm according to a reported method [24]. Each study was continued for a period of 72 h, during which the mean cumulative drug release, Q (μg/cm^2) across the membrane was calculated at each sampling time point (Table 1). The data obtained were subjected to ANOVA analysis. The graph of regressed Q vs. $t^{1/2}$ is shown in Fig. 1. At the completion of the study, the parafilm was removed and HCS was taken out. The drug remaining on the surface of the skin was removed by washing the surface three times with the medium and analysed for residual CLO. The residual washing solvent was carefully wiped off from the skin with a cotton swab. The skin was digested with 20 ml of toluene overnight at 40 °C and analysed for drug content in the skin spectrophotometrically. The results of the *in vitro* deposition study are shown in Table 2.

The flux values were calculated from the receptor fluids collected at the specified times using the equation [25].

$$J = V(dc/dt) \quad (1)$$

where J = flux of the drug across the membrane, V = volume of the receptor compartment and (dc/dt) = rate of change of concentration. The results are shown in Fig. 2. Each result represents the mean value of six experimental determinations.

3.6. Clinical studies

3.6.1. Trial methodology

The study involved eight leprosy inpatients (one female and seven male: aged 26–40 years) of the S.S.G. Hospital, Baroda, India. The patients had

been undergoing treatment with different drugs including CLO throughout the study and had been suffering from leprosy for six months to three years before the study. The four skin lesions selected for the study were located on the forearm, chest and stomach region which were marked and measured for their dimensions prior to the study. In a double blind design, a dose of 0.5 g of all the three formulations, (P, CLO6 and CLO7) was applied daily to each patient on three different premarked areas, after cleaning the local part with luke warm water. The fourth lesion was kept as control throughout the study. The treatment was continued for 12 weeks and the progress was evaluated on a weekly basis by a group of physicians in terms of decrease in dimensions of the skin lesion and skin staining due to CLO. The responses were graded on a five point scale (Fig. 3) and the response to the preparations were analysed statistically (ANOVA). The skin examination data before and after 12 weeks treatment period are presented in Table 3.

Acknowledgement: The authors wish to acknowledge the C.S.I.R., New Delhi for its financial assistance.

References

1 Skalko, N.; Cajkovac, M.; Jalsenjak, I.: Int. J. Pharm. **85**, 97 (1992)
2 Patel, H. M.: U. K. Patent GB2, **143**, 433A (1984)
3 Egbaria, K.; Weiner, N.: Adv. Drug Del. Rev. **5**, 287 (1990)
4 Mezei, M.; in: Gregoriadis, G. (Ed.): Liposomes as Drug Carriers, Recent Trends and Progress, p. 663, Wiley, Chichester 1988
5 Gabrijelcic, V.; Sentjure, M.; Kristl, J.: Int. J. Pharm. **62**, 75 (1990)
6 Foldvari, M.; Jarvis, B.; Oguejiofor, C.: J. Controll. Rel. **27**, 193 (1993)
7 Patel, V. B.; Misra, A. N.: J. Microencap., in press
8 Skov, M. J.; Quigley, J. W.; Bucks, D. A. W.: J. Pharm. Sci. **86**, 1138 (1997)
9 Patel, H. M.: Biochem. Soc. Trans. **13**, 513 (1985)
10 Margalit, R.; Alon, R.; Linenberg, M.: J. Controll. Rel. **17**, 285 (1991)
11 Higuchi, T.: J. Pharm. Sci. **50**, 874 (1961)
12 Shah, V. P.; Elkins, J. S.; Williams, R. L.: Pharmacopeial Forum **19**, 5048 (1993)
13 Betagari, G. V.: Drug. Dev. Ind. Pharm. **19**, 531 (1993)
14 Demel, R. A.; Dekruyff, B.: Biochem. Biophys. Acta. **457**, 109 (1976)
15 Masini, V.; Bonte, F.; Meybech, A.; Wepierre, J.: J. Pharm. Sci. **82**, 17 (1993)
16 Touitou, E.; Levi-Schaffer, F.; Dayan, N.; Alhaique, F.; Riccieri, F.: Int. J. Pharm. **103**, 131 (1994)
17 Egbaria, K.; Weiner, N.: Cosmet. Toil. **106**, 79 (1991)
18 Amin, P. D.; Fruitwala, M. A.: Drug Dev. Ind. Pharm. **20**, 1309 (1994)
19 Indian Pharmacopoeia Vol. II, p. A-145, Controller of Publications, Delhi 1996
20 Franz, J. J.: J. Invest. Dermatol. **64**, 190 (1975)
21 Barry, B. W.; in: Swarbrick, J. (Ed.): Dermatological Formulations-Percutaneous Absorption, 5. Ed., p. 234, Marcel Dekker Inc. New York and Basel 1983
22 Martin, B.; Watts, D.; Shroot, B.; Jamoulla, J. C.: Int. J. Pharm. **149**, 63 (1989)
23 Keshary, P. R.; Chien, Y. W.: Drug Dev. Ind. Pharm. **10**, 883 (1984)
24 Rao, G. V. H.; Thampi, P. P.; Thampi, C. S.: J. Inst. Chem. **62**, 127 (1990)
25 Yeung, D.; Nacht, S.; in: Bronaugh, R.; Maibach, H. (Eds,): Percutaneous – Absorption – Mechanisms – Methodology – Drug Delivery, p. 482, Marcel Dekker Inc., New York 1985

Received August 18, 1998
Accepted October 27, 1998

Prof. Dr. A. N. Misra
Pharmacy Department
Maharaja Sayajirao University of Baroda,
Post Box No. 51
Kalabhavan
Vadodara-390 001
India