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Liposomes based nasal delivery system of nifedipine: Development and characterization

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Abstract

Multilamellar liposomes bearing nifedipine were prepared using a conventional cast film method. The prepared liposomes were evaluated for release characteristics, in vitro, in situ bioadhesion and in vivo absorption following nasal administration. Charged components, stearylamine, dicetyl phosphate and some fusogenic/bioadhesive material were also incorporated into the liposomes. It was observed that positively charged liposomes possessed maximum bioadhesion while lysophosphatidylcholine liposomes showed considerable bioadhesion. In vivo experiments revealed that the nasal administration of liposomes eliminated hepatic first-pass metabolism and could maintain an effective drug concentration for prolonged periods of time with improved bioavailability.

Keywords: Nifedipine; Liposome; Bioadhesion; Nasal administration; In vivo-in vitro evaluation

1. Introduction

Systemic absorption of drugs through the nasal route is not a new concept. Several workers have reported that the systemic absorption of drugs through this route is either better than or comparable to that via other routes like the oral and parenteral pathways. The potential of this route has been studied for many drugs (Hussain et al., 1979; Champanale and Gries, 1984; Fisher et al., 1985; Morimoto et al., 1985; Duchateau et al., 1986; Harris et al., 1986; Chien et al., 1988; Daugherty et al., 1988; Lau et al., 1989; Lee et al., 1989).

The main problem with this route is rapid mucociliary clearance which results in decreased site of contact of the delivery system. Bioadhesive microspheres have been suggested to overcome this problem (Illum et al., 1987, 1988; Vyas et al., 1991). However, a few problems associated with such delivery systems like the obstruction of air passage and stability remain to be addressed.

Nifedipine, a calcium channel blocking agent, has received much attention for its utility in the management of cardiovascular disorders (Goodman and Gilman, 1985). It has been reported that the drug reaches the systemic circulation following nasal administration (Jain et al., 1990). In the present study, an attempt was made to develop a liposomal system of nifedipine for nasal administration which would not only improve bioavail-

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ability but also provide a sustained release profile of the drug.

2. Materials and methods

2.1. Materials

Nifedipine (Cipla Ltd, Bombay, India), egg lecithin (60% phosphatidylcholine), stearylamine, dicetyl phosphate, L-lysophosphatidylcholine (29% from egg yolk), Triton® X-100, 6-carboxyfluorescein, triolein (C_{18}, *cis*-9) (Sigma, St. Louis, USA), cholesterol (Loba Chemie, Bombay), ethanol, choloroform, acetone and methanol (Qualigens, a division of Glindia Ltd, Bombay) were obtained from the indicated sources.

2.2. Preparation of multilamellar liposomes

Liposomes were prepared using a conventional cast film method. Different molar ratios of egg lecithin, cholesterol and nifedipine were dissolved in 10 ml of a chloroform-methanol mixture (1:1) in a quickfit round-bottom flask. The contents of the flask were dried under controlled vacuum in a nitrogen atmosphere. The thin lipoidal film thus formed was hydrated in 10 ml of phosphate-buffered saline I.P. (pH 6.8) for 72 h. The flask was shaken during hydration with the help of a wrist action shaker (REMI, India Ltd). Liposomal size distributions were determined at 24-h intervals. Unentrapped drug was removed by washing the liposomal suspensions with phosphate-buffered saline. All the preparations were then stored at 4 ± 1°C. Liposomal systems containing charged and bioadhesive components such as stearylamine, dicetyl phosphate, lysophosphatidylcholine and triolein were also prepared.

2.3. Microscopic study of liposomes

The liposomal suspensions were subjected to size analysis under a microscope (Wild, Leitz, Germany) fitted with a calibrated ocular micrometer. The shape and lamellarity were also studied. 6-Carboxyfluorescein was used as a tracer to assess lamellarity.

2.4. Drug encapsulation efficiency determination

10 ml of freshly prepared liposomal suspension was centrifuged at 10000 rpm for 30 min (Eltek 40C super centrifuge). The supernatant was discarded and the pellet was suspended in 10 ml phosphate-buffered saline pH 6.8 (PBS). The resultant suspension was again centrifuged and the supernatant was discarded. Then, 1 ml of a 1% v/v aqueous solution of Triton X-100 was added to the pellet and the volume was made up to 10 ml with PBS. The amount of drug present in the resulting solution was estimated spectrophotometrically at 340 nm. The percent entrapment was calculated on the basis of the initial lipid weight.

2.5. In situ bioadhesion study

To investigate the bioadhesion of liposomal preparations, the rat mucosal experimental arrangement of Faraj et al. (1990) with minor modifications was used. The liposomal suspension (4 ml) was placed in a water-jacketed beaker maintained at 37°C. The suspension was circulated through the nasal cavity of the rat at a flow rate of 0.7 ml/min using a peristaltic pump for 10 min. The number of liposomes/mm^3 in the original suspension and in the perfusate, i.e., the suspension collected after 10 min circulation, was determined with the help of a haemocytometer. The difference between these numbers was taken as the number of liposomes which adhered to the site of the nasal mucosa. Bioadhesion study was performed on incised rats (surgical procedure of Huang et al. (1985)) and for each study a different albino rat ($\cong$ 250 g) was used.

2.6. Stability studies

Stability studies were conducted by incubating liposomes for 1 h at different pH values. Average size, liposome number and percentage leaching before and after incubation of liposomal suspension(s) were determined. The number of liposomes was counted with the help of the haemocytometer.

2.7. In vitro release rate study

In vitro drug release from selected liposomal systems was determined using dialysis bags (Sigma, USA). A schematic diagram of the apparatus is shown in Fig. 1. The dialysis tube was washed following the method described by the supplier (Sigma). Then, one end of the tube was tied and 5 ml of liposomal suspension was filled. The other end of the tube was also tied with a thread. The dialysis bags containing liposomal preparations were suspended vertically into a beaker containing 100 ml of PBS. The contents of the beaker were stirred using a magnetic stirrer, the temperature being maintained at 37 ± 1°C. The samples were withdrawn periodically with the help of a micropipette and replaced with fresh PBS. The amount of drug was determined by observing the absorbance at 340 nm.

2.8. In vivo evaluation

In vivo performance study was conducted on two groups of rabbits, six rabbits in each group, in a crossover design. The rabbits were anesthesized with pentobarbitone sodium (40 mg/kg body weight, intraperitoneally) prior to administration of developed system(s).

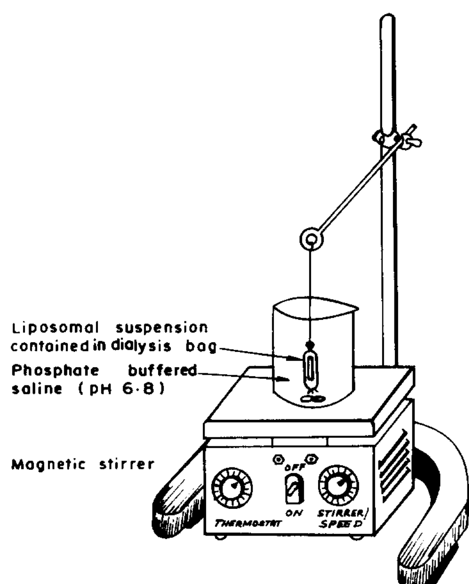

Fig. 1. Schematic diagram representing in vitro release rate study.

Table 1
Percentage nifedipine entrapment and average liposomal size in different liposomal systems

No.	Liposome code	Egg lecithin : chol : a or b or c or d: nifedipine	% drug entrapment	Average liposomal size (μm)
1	Le_1	5:1:0:0.5	32.0 ± 0.5	5.2 ± 0.96
2	Le_2	6:1:0:0.5	36.0 ± 0.8	8.4 ± 1.1
3	Le_3	7:1:0:0.5	43.5 ± 1.2	10.8 ± 1.3
4	Le_1SA [a]	5:1:2:0.5	33.5 ± 1.1	5.5 ± 1.0
5	Le_2SA [a]	6:1:2:0.5	37.1 ± 0.9	8.6 ± 1.2
6	Le_3SA [a]	7:1:2:0.5	45.0 ± 1.7	10.8 ± 1.4
7	Le_1DCP [b]	5:1:2:0.5	35.0 ± 1.0	5.8 ± 1.1
8	Le_2DCP [b]	6:1:2:0.5	37.9 ± 1.3	8.9 ± 1.3
9	Le_3DCP [b]	7:1:2:0.5	45.8 ± 1.8	10.9 ± 1.5
10	Le_3LPC [c]	7:1:0.1:0.5	41.0 ± 1.5	10.84 ± 1.4
11	Le_3TO [d]	7:1:1:0.5	44.0 ± 1.7	10.88 ± 1.5

a, stearylamine (SA); b, dicetyl phosphate (DCP);
c, lysophosphatidylcholine (LPC); d, triolien (TO);
Le, neutral liposomes;
LeSA, SA-based liposomes;
LeDCP, DCP-based liposomes;
LE_3LPC, LPC-based liposomes; Le_3TO, TO-based liposomes;
± indicates ± s.D. ($n = 3$).

A dose of 0.8 ml liposomal suspension(s) was administered through one nostril with the help of a micropipette. Blood samples were collected periodically through the marginal ear vein. After centrifugation 0.5 ml of the plasma was separated and analysed by the HPLC method described by Miyayaki et al. (1984). After each administration, a 7 day washout period was allowed. Similarly to the reference study, the plasma level achieved after the nasal administration of a nifedipine solution containing an equivalent amount of drug was determined.

3. Results and discussion

Different liposomal systems were prepared using the cast film method and characterised (Table 1). The average size of liposomes in formulations Le_1, Le_2 and Le_3 was found to be 5.2, 8.4 and 10.8 μm, respectively, indicating that the average size increased with increasing phospholipid con-

centration. Liposomes were spherical in shape and were found to be multilamellar. The lamellae in formulations Le_1 and Le_2 were clearly visible. Incorporation of 6-carboxyfluorescein further confirmed their multilamellar nature. The distance between two consecutive lamellae appeared to be constant.

3.1. Effect of lipid concentration on drug entrapment

The effect of lipid concentration on drug entrapment was noted (Table 1). The percent drug entrapment was found to be 32.0 ± 0.5, 36.0 ± 0.8 and 43.5 ± 1.2 for preparation Le_1, Le_2 and Le_3, respectively. The study indicated that drug entrapment increases with increasing phospholipid concentration. This could be ascribed to the lipophilic nature of drug.

3.2. Effect of charge

Charged components stearylamine (positive) and dicetyl phosphate (negative) were incorporated into liposomes. Charge incorporation resulted in a slight increase in average liposomal size (0.2–0.6 μm) and percent drug entrapment (Table 1). Stearylamine increased the percent entrapment 32.0 ± 0.5, 36.0 ± 0.8 and 43.5 ± 1.2 to 33.5 ± 1.1, 37 ± 1.7 and 45.0 ± 1.7 in preparations Le_1, Le_2 and Le_3, respectively, whereas dicetyl phosphate increased the percent entrapment relatively more, i.e., to 35.0 ± 1.0, 37.9 ± 1.3 and 45.8 ± 1.8 for preparation Le_1, and Le_2 and L_3, respectively. The increase in entrapment may possibly be attributed to interlamellar repulsion and ion pair formation between the drug and charged components. Although apparent differences in percent drug entrapment were noted, these differences are, however, statistically insignificant ($P < 0.05$).

3.3. Effect of LPC

Fusogenic material, lysophosphatidylcholine (LPC), was incorporated into preparation Le_3. There are reports indicating the use of LPC as liposomal component and nasal absorption enhancers (Finkelstein and Weissman, 1981; Illum *et al.*, 1989). Incorporation of LPC decreased the percent drug entrapment of Le_3 (from 43.5 ± 1.2 to 41.0 ± 1.5, the difference being statistically insignificant ($P < 0.05$) which may be due to increased permeability of liposomes. Triolein, a bioadhesive material, was found to increase the percent entrapment from 43.5 ± 1.2 to 44.5 ± 1.7

3.4. In situ bioadhesion study

An *in situ* bioadhesion study was performed (Table 2). For preparations Le_1, Le_2 and Le_3, the

Table 2
In situ bioadhesion study in rats

No.	Liposomal code	Number of liposomes/mm^3 in applied liposomal suspension ($\times 10^5$)	Number of liposomes/mm^3 in collected liposomal suspension ($\times 10^5$)	% bioadhesion of liposomes to nasal mucosa
1	Le_1	6.0	4.0	33.0 ± 3.2
2	Le_2	5.5	3.5	36.3 ± 2.7
3	Le_3	4.8	3.6	35.4 ± 3.5
4	Le_1SA	5.5	0.6	89.1 ± 4.3
5	Le_2SA	5.2	0.5	90.4 ± 5.0
6	Le_3SA	4.4	0.4	90.9 ± 4.8
7	Le_1DCP	5.2	5.1	1.92 ± 0.21
8	Le_2DCP	3.9	3.9	no bioadhesion
9	Le_3DCP	3.4	3.4	no bioadhesion
10	Le_3LPC	4.5	1.1	75.5 ± 3.7
11	Le_3To	4.4	1.8	59.1 ± 2.8

± indicates S.D. where $n = 4$.

Table 3
Stability study of different liposomal systems at different pH values

Number and average size before and after incubation	pH	Le_3			Le_3SA			Le_3LPC			Le_3TO		
		No./mm^3 ($\times 10^5$)	Av. size (μm)	% leaching	No./mm^3 ($\times 10^5$)	Av. size (μm)	% leaching	No./mm^3 ($\times 10^5$)	Av. size (μm)	% leaching	No./mm^3 ($\times 10^5$)	Av. size (μm)	% leaching
Initial liposomal count, average size and % leaching	7.0	4.8	10.8	4.5	4.4	10.88	3.0	4.5	10.84	10	4.4	10.88	3.0
Final liposomal count average size and % leaching	2.0	3.5	11.52	5.2	3.9	11.0	4.5	3.8	14.20	13	3.9	11.75	4.1
	5.0	3.9	11.70	5.0	3.7	11.3	5.2	3.4	15.20	14.2	4.2	11.88	4.5
	6.8	4.6	11.1	4.7	4.1	10.95	3.2	3.3	15.0	15.5	4.3	11.20	4.3
	9.0	3.6	11.43	5.4	3.6	11.2	5.6	2.3	17.3	14.3	4.3	11.99	4.9

Each value is the mean of three observations.

percent bioadhesion was recorded to be 33.3 ± 3.3, 36.3 ± 2.7 and 35.4 ± 3.5, respectively. Maximum bioadhesion was demonstrated by the liposomal preparation containing stearylamine. This can be attributed to the fact that mucin, the main constituent of mucus produced by goblet cells of nasal mucosa, carries a significant negative charge at physiological pH due to the presence of sialic acid and sulphate residues (Gandhi and Joseph, 1988). This charge contributed toward rapid electrostatic bioadhesion in the case of stearylamine-containing liposomes, while in the case of DCP-containing liposomes, repulsion resulted, reflecting less bioadhesion. Maximum actual bioadhesion was noted for Le_3SA (90.9 ± 4.8%), while no bioadhesion was shown by preparations Le_2DCP and Le_3DCP. Liposomes containing LPC (Le_3LPC) and triolein (Le_3TO) exhibited considerable bioadhesion.

3.5. *Stability study of liposomes*

The formulations Le_3, Le_3SA, Le_3LPC and Le_3To were subjected to stability studies (Table 3). The study revealed that all the preparations were stable at pH 6.8, i.e., the pH of the nasal cavity. For each pH and liposome type after 1 h incubation, a marginal increase in average size and decrease in number of liposomes/mm^3 were noted, possibly due to clumping of liposomes. Incorporation of the charged component stearylamine increased the stability. This is probably due to repulsion, which reduced the chances of liposome-liposome contact. Preparation Le_3To was found to be more stable than Le_3LPC.

3.6. *In vitro drug release study*

The drug release study of products Le_3, Le_3SA, Le_3LPC and Le_3To was performed for 12 h using dialysis bags (Fig. 2). It was observed that Le_3LPC exhibited maximum release (65.7 ± 3.3% in 12 h). This could be attributed to the fact that lysophosphatidylcholine enhances the permeability of the liposomal membrane. Le_3SA and Le_3To were found to release 42.4 ± 5.3 and 57.2 ± 3.5% drug in 12 h, respectively. Le_3SA released more drug than Le_3, probably due to the stretching of lamellae because of interlamellar repulsion in the case of Le_3SA.

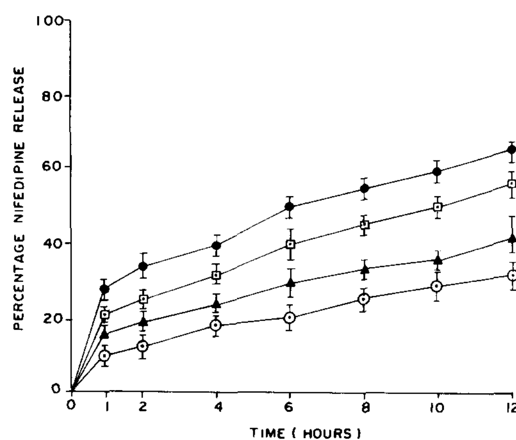

Fig. 2. In vitro release rate plot of different liposomal systems. Le_3 (○——○). Le_3Sa (△——△). Le_3TO (□——□) and Le_3LPC (●——●). Bar at data point indicates ±S.D. (n = 4) (temperature 37 ± 0.5°C).

3.7. *In vivo study*

To elucidate the *in vivo* performance of developed system(s), various pharmacokinetic parameters and plasma concentration-time profile were determined (Table 4 and Fig. 3). It was found that nifedipine solution exhibited the highest plasma level (600 ng/ml in the first hour,

Table 4

C_{max}, t_{max} and AUC_{0-12} values after nifedipine nasal treatments

Treatment	AUC_{0-12} [a] ±S.E. (ng h ml^{-1})	t_{max} (h)	C_{max} (ng ml^{-1})
Aqueous nifedipine solution	3144 ± 140	1	600
Stearylamine-based liposomal system (Le_3SA)	3247 ± 210	2	303
Lysophosphatidyl-choline-based liposomal system (Le_3LPC)	4193 ± 315	2	409
Triolein-based liposomal system	2549 ± 170	2	250

[a] Calculated by trapezoidal rule.

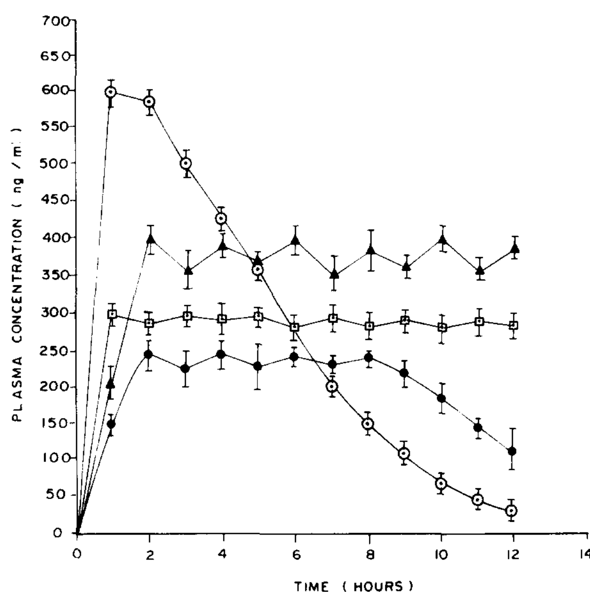

Fig. 3. Plasma concentration-time profile after nasal administration of nifedipine solution (⊙——⊙). Le_3LPC (▵——▵), Le_3SA (⊡——⊡) and Le_3TO (●——●) in rabbits. Bar at data point indicates ±S.D. ($n = 6$).

AUC_{0-12} 3144 ± 140 ng h ml^{-1}), however the plasma level declined rapidly. Also, C_{max} reached the toxicity level. In contrast, liposomal formulations showed a rapid increase in plasma level for the initial 1–2 h and then a constant plasma level for other 8–10 h. The plasma concentration did not exceed the effective level and remained between 250 and 450 ng/ml throughout. Thus, C_{max} in the case of developed system(s) remained within the therapeutic range. The peaks and valleys in the plasma profile of various formulations are probably due to the gradual fusion of liposomes with nasal mucosa and subsequent pulsed release of the drug. The initial rapid increase in plasma level is presumably due to the absorption of free drug present over liposomal surface and in the vehicle. Formulation Le_3LPC showed the highest C_{max} value, probably due to high fusogenicity of the system. Le_3SA exhibited an almost constant plasma level and thus can be preferred over other systems to avoid fluctuations.

This study has demonstrated that the liposomal system(s) can be effectively used to control the release of drug. Thus, nifedipine-bearing multilamellar liposomes can be employed successfully to attain a constant plasma profile of the same. The system containing stearylamine, in particular, is a potential delivery system which can overcome problems like mucocilliary clearance and nasal obstruction associated with other systems. Such system(s) could be used for drugs with a first-pass effect and instability problems and hence in achieving controlled release.

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