Drug Development and Industrial Pharmacy, 24(7), 671–675 (1998)

COMMUNICATION

Brain Drug Delivery System Bearing Dopamine Hydrochloride for Effective Management of Parkinsonism

Naveen K. Jain, A. C. Rana, and Sanjay K. Jain*

Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Doctor Harisingh Gour Vishwavidyalaya, Sagar-470 003 (M.P.), India

ABSTRACT

Dopamine hydrochloride bearing positively charged small liposomes was prepared by sonicating the multilamellar vesicles. These vesicles were characterized for their physical attributes (shape, size, charge, drug entrapment efficiency, and drug leakage). The drug release kinetics from the liposomes were also studied and found to be Fickian-type diffusion. In vivo performance of the drug-entrapped liposomes was assessed by periodic measurement of drug- (chlorpromazine) induced catatonia in Sprague–Dowley rats. These results were compared with the plain dopamine HCl and levodopa preparations as well with the marketed formulation of levodopa containing carbidopa (Syndopa®). These studies revealed that the dopamine can be effectively delivered to the brain by incorporating it into liposomes, and its degradation in circulation can also be protected. The results of liposomal formulation were found to be superior compared to plain levodopa as well as Syndopa.

INTRODUCTION

Drug delivery to the brain requires advances in both drug discovery and a delivery system. The management of brain disorders with presently available therapeutic systems is very difficult because insufficient drug reaches the brain due to the presence of the highly lipophilic blood brain barrier (BBB). The lipophilic nature of the BBB permits only small lipid-soluble drug to pass through this barrier (1). Therefore, practical strategies are required to develop a system that can facilitate the transport of poorly permeable drugs across the BBB for effective management of such disorders. Because of the presence of a tight junction of endothelial cells in BBB (2), drug access to brain interstitial is via only one or two pathways, i.e., lipid-mediated transport, or catalyzed

*To whom correspondence should be addressed.

transport via a carrier- or receptor-mediated process. Certain specialized carrier transport systems and specific receptors are present in the BBB that mediate the brain's uptake of circulating nutrients and drugs (1,3).

Various strategies are available to deliver drug into the brain. Some are based upon the nature (lipophilicity) and size of the drug molecule. The lipid-soluble small molecules readily cross the BBB and lipid-insoluble molecules are impermeable. Therefore, several attempts have been made to transport molecules through the BBB, i.e., by conversion to a nutrient such as a prodrug or lipid-soluble prodrug, modification of drug to enhance lipophilicity and cerebrovascular permeability, or use of carrier- or receptor-mediated transport (1). Kim et al. (4) reported that the transport of small molecules through biological membranes such as the BBB was facilitated using liposomes. It is assumed that positively charged small unilamellar vesicles may cross the BBB via either carrier- or receptor-mediated transcytosis system. Dopamine HCl is an essential agent used to manage parkinsonism; as such it does not cross the BBB and has a very short biological half-life (5). Therefore, an attempt was made to deliver dopamine HCl to the brain using liposomes as carriers for effective management of parkinsonism.

MATERIALS AND METHODS

Materials

Dopamine hydrochloride (Spectrochem Pvt. Ltd., Bombay, India) and egg phosphatidylcholine, cholesterol, and stearylamine (Sigma Chemical Co., St. Louis, MO) were used. Chloroform, methanol, and all other chemicals were of analytical grade and used as received.

Preparation of Liposomes

Small unilamellar vesicles were prepared using the method reported by Saunder et al. (6). Egg phosphatidylcholine (EPC), cholesterol (CH), and stearylamine (SA) were dissolved in 6:3:1 molar ratio in a mixture of chloroform:methanol (2:1). A dried thin film of this solution was formed on the inner wall of a round-bottom flask using a rotary flask evaporator (Buchtype, Yarko, Sci. Co., Bombay, India). Then, dried thin film was hydrated with phosphate buffer saline (PBS) of pH 4.5 containing dopamine HCl (25 mg/ml) for 1 hr and allowed to stand 3–4 hr in the dark for complete swelling of the vesicles. The formed vesicles were centrifuged (10,000 × g), washed three to four times with PBS (pH 4.5), and resuspended in PBS (pH 4.5) containing drug (25 mg/ml). This liposomal suspension was sonicated for 1 min in an ice bath. The suspension was then centrifuged and washed three times with PBS (pH 4.5). These drug-containing liposomes were characterized for shape, size, charge, entrapment efficiency, and drug leakage (Table 1).

Table 1

Characterization of Formulation

Parameter	Results
Shape	Spherical
Size	0.2 μm
Charge	Positive
Entrapment efficiency	40.5%
Drug leakage in 24 hr	6.8%

In Vitro Drug Release

The in vitro drug release from the formulated products was studied using Franze diffusion cell (Crown Glass Co., Somerville, NY) and PBS (pH 4.5) as diffusion media. The cellophane membrane was clamped between the donor and receptor compartment of the cell. The preparation was kept over the cellophane membrane and the temperature of the receptor fluid was kept at 37 ± 1°C by a circulating water bath. The samples (1 ml) were withdrawn at each scheduled time interval and immediately replaced with fresh PBS. The drug concentration in the samples was determined spectrophotometrically at 280 nm using the method reported in USP (7).

In Vivo Performance Evaluation

The in vivo performance of liposomal product was assessed by measuring the reduction in the degree of drug-induced catatonia in Sprague–Dowley rats (8). The performance of the products was also compared with plain dopamine HCl solution, plain levodopa solution, and marketed levodopa preparation containing carbidopa (Syndopa®).

Study Design

Thirty animals were selected, weighed, and divided into five groups, each having six animals. The first group's animals were kept as control (without drugs), and the second through fifth group's animals received intraperitonially (i.p.) dopamine HCl solution (100 mg/

kg), levodopa solution (100 mg/kg), Syndopa (levodopa + carbidopa; 50 mg/kg), and formulated liposomal preparation containing dopamine HCl (100 mg/kg), respectively. The chlorpromazine (5 mg/kg) was injected intraperitonially to all animals 30 min after the drug treatment. The reduction in the degree of catatonia was then recorded periodically.

Method

The reduction in degree of catatonia was assessed by scoring the animals periodically. Difficulty in moving and changing the posture by the animals was evaluated by alternately placing the paw of the rat on a 3-cm- and 9-cm-high block. Scoring was done in the following manner.

Stage I: Rat moved normally when placed on observation table, scored zero.

Stage II: Rat moved only when touched or pushed, scored 0.5.

Stage III: Rat placed on the table with front paw set alternately on a 3-cm-high block; if animal failed to correct the posture in 10 sec, scored 0.5 for each paw with a total of 1 for this stage.

Stage IV: Rat placed on the table with front paw set alternately on a 9-cm-high block; if animal failed to correct the posture in 10 sec, scored 1 for each paw with a total score of 2 for this stage.

Table 2

Score of Reduction in Degree of Catatonia of Different Formulations

Group No.	Treatment	Dose (mg/kg)	Individual Score Time Interval (min)							
			0	15	30	45	60	90	120	150
1	Chlorpromazine	5	0.0	0.0	0.5	1.5	1.5	3.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	3.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	3.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	3.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	3.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	3.5	3.5	3.5
2	Dopamine HCl solution	100	0.0	0.0	0.5	1.5	1.5	1.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	1.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	1.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	1.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	1.5	3.5	3.5
			0.0	0.0	0.5	1.5	1.5	1.5	3.5	3.5
3	Levodopa solution	100	0.0	0.0	0.0	0.5	0.5	1.5	1.5	1.5
			0.0	0.0	0.0	0.5	0.5	1.5	1.5	1.5
			0.0	0.0	0.0	0.5	0.5	1.5	1.5	1.5
			0.0	0.0	0.5	0.5	1.5	1.5	1.5	3.5
			0.0	0.0	0.5	0.5	1.5	1.5	1.5	3.5
			0.0	0.0	0.0	0.5	0.5	1.5	1.5	1.5
4	Syndopa	25	0.0	0.0	0.0	0.5	0.5	1.5	1.5	1.5
			0.0	0.0	0.0	0.5	0.5	0.5	1.5	1.5
			0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5
			0.0	0.0	0.0	0.5	0.5	1.5	1.5	1.5
			0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5
			0.0	0.5	0.5	0.0	0.5	0.5	0.5	0.5
5	Lip. D.C.	100	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5
			0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5
			0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5
			0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5
			0.0	0.0	0.0	0.0	0.0	0.5	0.5	1.5
			0.0	0.0	0.0	0.0	1.0	0.5	0.5	0.5

Lip. D.C.: Dopamine hydrochloride-bearing liposomes.

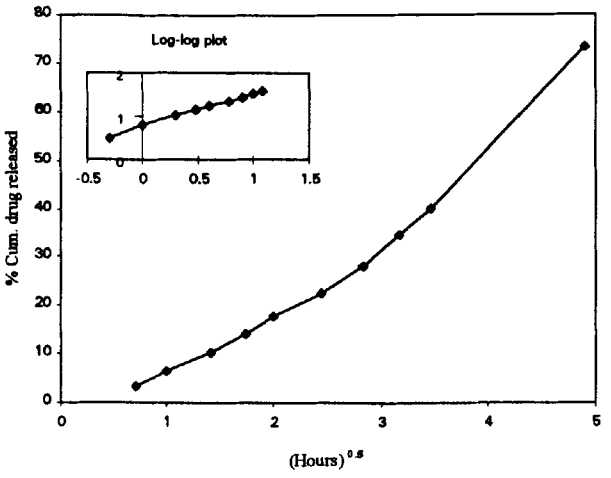

Figure 1. In vitro drug release profile of drug-loaded liposomal system.

Thus, for a single rat, the maximum possible score was 3.5, which revealed total catatonia. The onset and severity of catatonic response in all groups were compared by observing the catatonia at 0, 15, 30, up to 150 min (Table 2).

RESULTS AND DISCUSSION

Dopamine HCl-entrapped liposomes were prepared using the method reported by Saunder et al. (6). The small drug-entrapped liposomes were separated by centrifugation at 10,000 × *g* for 10 min. These liposomes were spherical and positively charged, having an average size of 0.2 μm and 40.5% drug encapsulation efficiency. The drug leakage study revealed that only 6.8% drug leaked in 24 hr if suspended in PBS pH 4.5. The drug-loaded liposomal system (Lip. D.C.) was studied for in vitro drug release using the Franz diffusion cell and PBS pH 4.5 diffusion media. The in vitro drug release studies revealed that the drug released from the liposomes followed Fickian diffusion kinetics because the diffusional release exponent was found to be 0.5 from the log cumulative drug release versus log time curve. Therefore, a linear relationship was obtained between percent cumulative drug release versus time$^{0.5}$ (Fig. 1).

The in vivo performance of the liposomal product was evaluated by measuring the reduction in the drug-induced catatonia in Sprague–Dowley rats. The results were compared with the dopamine HCl solution, levodopa solution, and marketed levodopa preparation containing carbidopa (Syndopa). In Table 2, the scores of animals of each group after 15 min of drug administration are summarized. Results revealed that dopamine HCl solution did not show any reduction in degree of catatonia (maximum score 3.5 after 150 min), and animals from the third and fourth groups which received levodopa solution and Syndopa i.p. showed partial reduction in degree of catatonia. The liposomal dopamine formulation completely abolished catatonia in animals from the fifth group (maximum score 0.5 after 150 min in five animals and 1.5 in one animal). Moreover, in animals from the fifth group, the score was zero until 60 min, then the score increased to a maximum of 0.5, suggesting effective action of the drug-bearing liposomes (Table 3). Dopamine as such does not cross the BBB and did not elicit any effect on drug-induced catatonia. Dopamine incorporated into liposomes showed significant effect because liposomes facilitated the transport of drug through the BBB and protected the drug from inactivation. Therefore, almost complete abolition of drug-induced catatonia was seen with liposomes bearing dopamine. It is also reported that levodopa crosses the BBB but undergoes peripheral decarboxylation; therefore, plain solution showed partial reduction in drug-induced catatonia. The marketed product Syndopa containing levodopa and carbidopa showed significant effect because the peripheral decarboxylation of levodopa was inhibited by carbidopa.

In conclusion, it can be stated that the dopamine HCl can effectively be delivered to the brain by incorporat-

Table 3

Effect of Different Formulations on the Drug-Induced Catatonia in Rats

Action	Dopamine HCl Solution 100 mg/kg	Levodopa Solution 100 mg/kg	Syndopa 25 mg/kg	Lip. D.C. 100 mg/kg
No reduction	6	2	None	None
Partial reduction	None	4	3	1
Complete reduction	None	None	3	5

n = 6. Lip. D.C.: Dopamine hydrochloride-bearing l iposomes.

ing it into the positively charged small liposomes. Hence, parkinsonism can be managed effectively by using dopamine HCl-bearing liposomes. However, detailed pharmacokinetic and clinical studies are necessary to determine the drug concentration in various body fluids and to establish its effectiveness in the management of parkinsonism.

ACKNOWLEDGMENTS

The authors are thankful to Prof. V. K. Dixit, Head, Department of Pharmaceutical Sciences, Doctor Harisingh Gour Vishwavidyalaya, Sagar (M.P.), India, for providing necessary facilities in the completion of this work.

REFERENCES

1. W. M. Pardridge, Drug Delivery, 1, 83 (1993).
2. B. V. Zlokovic, J. Controlled Release, 13, 185 (1990).
3. M. W. Brightman, Exp. Eye Res., 25(suppl.), 1 (1977).
4. S. Kim, S. Scheerer, M. A. Geyer, and S. B. Nowell, J. Infect. Dis., 162, 7915 (1990).
5. Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 8th ed., Vol. I, J. (G. Hardman, L. E. Limbird, P. B. Molinoff, R. W. Ruddon, and A. G. Gilman, eds.) Pergamon Press, New York, 1991, p. 187.
6. L. Saunder, J. Perrin, and D. B. Gammaok, J. Pharm. Pharmacol., 14, 507 (1962).
7. *United States Pharmacopeia*, 23rd ed., NF 18, Asian Edition, The United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, p. 549.
8. S. K. Kulkarni, *Hand Book of Experimental Pharmacology*, Vallabh Prakashan, New Delhi, India, 1990, p. 72,