Drug Development and Industrial Pharmacy, 23(8), 827-830 (1997)

COMMUNICATION

Development of a Liposome Based Contraceptive System for Intravaginal Administration of Progesterone

Sanjay K. Jain,* Ranjit Singh, and Balram Sahu

Pharmaceutics Laboratory, Department of Pharmaceutical Sciences, Doctor Harishingh Gour Vishwavidyalaya, SAGAR (M.P.) 470 003, INDIA

ABSTRACT

Progesterone bearing liposomes were prepared by the cast film method and characterized for various physical attributes. The liposomes could encapsulate nearly 98% of the drug. The progesterone bearing liposomes were incorporated into polyacrylamide gel and the drug content was determined. The in vitro drug diffusion across she-goat vaginal membrane from the liposomal formulation was found to follow near zero order kinetics. The progestational activity of liposomal and control gel was assessed by monitoring the effect on the formation of corpora lutea. It was observed that both the formulations inhibit the formation of corpora lutea and thus, exhibited progestational activity but the effect of liposomal preparation was found to be greater and prolonged as compare to control gel.

INTRODUCTION

The effectiveness of the vaginal cavity as a site of drug administration for systemic and local effects has been well established (1-2). A wide variety of organic and inorganic compounds, penicillin, sulfa drugs, proteins, nonoxynol-9, and methadone are known to be well absorbed from the vaginal mucosa (3).

The vaginal route of administration of drugs has some advantages over other routes such as ease of application, allow complete privacy for women, absence of side effects, avoidance of hepatic "first pass metabolism" and gastrointestinal incompatibility (4-5).

Progesterone, a potent contraceptive, is extensively metabolized on oral administration because of "first pass metabolism" (6). The drug could be administered using various routes, however, the intravaginal route of administration is considered to be most suitable and effective. An assortment of intravaginal devices for controlled delivery of progesterone is available, but, being mechanical devices they cause discomfort to the patient. Thus, it was thought worthwhile to develop a bio-com-

*To whom correspondence should be addressed.

patible formulation which could effectively deliver progesterone via intravaginal route for an extended period of time. Liposomes containing progesterone were prepared and evaluated for various in vitro attributes and in vivo performance.

MATERIALS AND METHODS

Materials

Egg lecithin, dicetyl phosphate (Sigma Chemicals Co. St Louis MO), cholesterol (Loba Chemie, Bombay, India). Progesterone was generously supplied by Unichem Laboratories Ltd., Bombay, India. Other chemicals used were of analytical reagent grade.

Preparation of Liposomes

Progesterone bearing multilamellar liposomes were prepared using cast film method reported by Bangham (7). Drug, egg lecithin, cholesterol, dicetyl phosphate (2:6:3:1, weight fraction ratio) were dissolved in cholorform:methanol (2:1). The solution was evaporated in a round bottom flask under a stream of nitrogen to form lipid film which was hydrated with saline phosphate buffer (pH 7.4) under constant vortexing for an hour. The dispersion was kept for further 2 hr at room temperature for complete hydration of liposomes. The dispersions were centrifuged, lyophilized, and transferred to amber colored glass vials having nitrogen atmosphere and stored in a refrigerator.

Preparation of Liposomal and Control Gels

Hydrogels were prepared by the dispersing 1% w/v poly(acrylamide) in water containing 3% w/w glycerine. The gel was allowed to swell at 45 ± 2° under constant stirring. A calculated quantity of progesterone bearing lyophilized liposomes was mixed thoroughly with the hydrogel followed by gentle stirring to produce 1% w/w concentration of drug in the gel.

Similarly, polyacrylamide control hydrogel bearing progesterone (1% w/w) was prepared by mixing polymer and the drug and allowed to swell in warm water containing 3% w/w glycerin under continuous stirring.

Size Distribution

Liposomes were mounted on a glass slide and viewed under a phase contrast microscope (Wildleitz, Germany). The size was determined using ocular and stage micrometer.

Encapsulation Efficiency

The liposomal dispersion was centrifuged at 10,000 rpm (Eltek Centrifuge) for 30 min and the sediment was washed with saline phosphate buffer (pH 7.4). Then, the liposomes were lysed with 1 ml solution of Triton X-100 (1% w/v) and volume was made to 10 ml by methanol. The resulting solution was assayed spectrophotometrically at 252 nm against respective blank solution.

Drug Content in the Gels

The progesterone content in gels was determined by diluting an accurately weighed amount of gel with an excess of distilled water. 1 ml 1% w/v Triton X-100 solution was added in case of gel containing progesterone bearing liposomes. The drug content was determined in the solution by measuring the absorbance against respective blank solution at 252 nm.

In Vitro Vaginal Permeation Studies

Fresh she-goat vagina was procured, cut open vertically and vaginal mucosa was separated. It was prepared for study using the method reported by Kabadi and Chien (8) for determination of transvaginal permeation of drug.

Procedure

A piece of prepared vaginal membrane was mounted on a franz diffusion cell (Crown glass co. NJ, USA). The mucosal side of vagina was exposed to ambient conditions, while the serosal side was continuously bathed with saline phosphate buffer (pH 4.5) containing 40% w/w PEG-400 to improve solubility of drug and maintain the sink condition. The temperature of the receiver solution was kept constant at 37 ± 1° using a circulatory water bath.

The cell was allowed to stand overnight prior to the start of the experiment. This allowed sufficient time for equilibration with temperature and humidity of the surrounding environment. An accurately weighed quantity of formulation was placed over the vaginal mucosa in the donar compartment of the diffusion cell.

Samples (1 ml each) were withdrawn periodically from the receiver compartment and replaced immedi-

ately with same volume of fresh saline phosphate buffer (pH 4.5) containing 40% PEG 400. The drug concentration in these samples was determined spectrophotometrically at 252 nm.

Evaluation for Progestational Activity

Progestational activity of liposomal and control gel was measured by measuring the formation of corpora lutea (9). The activity of liposomal gel was compared with that of control gel.

Female mature albino rats (100–150g) were divided into three groups (42 rats in each). The rats of group I were treated with liposomal gel and the rats of group II were treated with control gel intravaginally. The rats of the third group were kept as control. After the 48 hours of application of formulation six rats from each group were sacrificed every day for seven days. The mean number of corpora lutea per treatment present were recorded by histological examination of ovaries isolated from these rats. The mean numbers of corpora lutea per treatment group were calculated (Table 1).

RESULTS AND DISCUSSION

Progesterone bearing multilamellar liposomes were prepared using the cast film method. These liposomes were characterized for shape, size, and encapsulation efficiency. The average size of liposomes were found to be 6.73 ± 0.85 μm.

The encapsulation efficiency of liposomal suspension was found to be 97.8 ± 1.1%. The high entrapment of

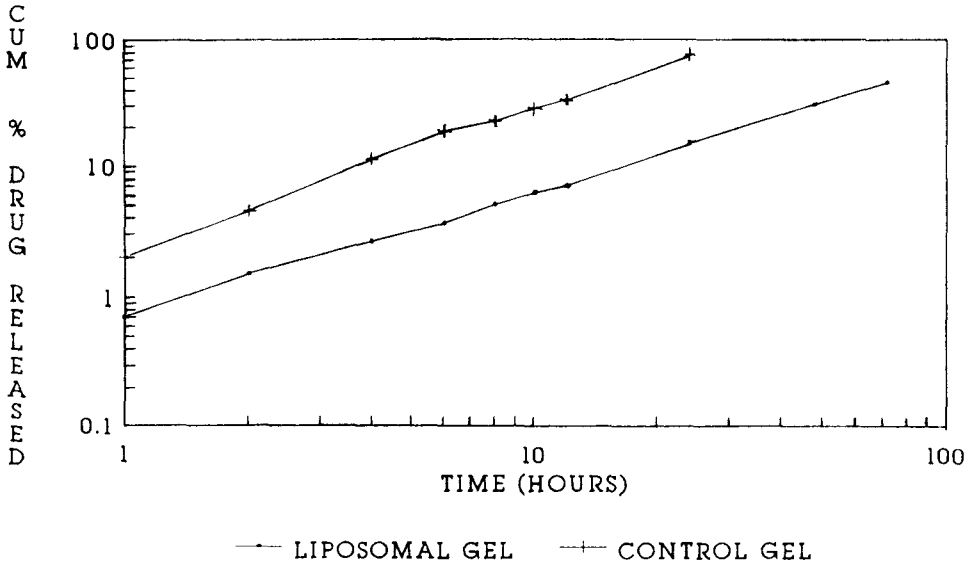

Figure 1. In vitro release profile of progesterone through she-goat vaginal membrane. (Log-log plot.)

Table 1

Inhibition of Luteinization in the Rat Ovary by Liposomal Gel and Control Gel

TREATMENT	No. of Rats	Daily Dose (ml)	Mean Body Weight (gm)	No. of Rats with C.L.	Mean No. of C.L.	Inhibition (%)	Duration
I. None	6	—	125	5 ± 1	8.5 ± 0.6	—	—
II. Control Gel	6	0.5	120	3 ± 2	4.6 ± 0.4	46 ± 8	2 days
III. Liposomal Gel	6	0.5	135	1 ± 1	1.2 ± 0.8	85 ± 9	7 days

C.L.: Corpora Lutea.

the progesterone into liposomes may be attributed to the lipoidic nature of the drug.

Hydrogel was prepared using polyacrylamide and liposomal pellets were mixed in quantities which approximately 1% w/w incorporated drug into the gel. One percent plain drug (without liposome) was incorporated into the gel and used as control. Drug content of control and liposomal gel were determined and found to be 95.3 ± 1.6 and 94.5 ± 1.3%, respectively.

The prepared liposomal products were subjected to release profile studies. The in vitro vaginal permeation study revealed that the drug permeation across vaginal membrane from control as well as liposomal products follows near zero order kinetics (permeation exponent was found to be 1.1 and 1.05 determined using the slope of log cumulative drug permeated v/s log time plot, Figure 1) (10). A lag time of 35 min for liposomal gel and 20 min for control gel was observed. This higher lag time with liposomal product may be due to the formation of liposomal depot in the vaginal membrane as a consequence the permeation of drug across the vaginal membrane was retarded resulting a prolonged release.

In vivo results revealed that the formation of functioning corpora lutea was decreased in treated animals (Table 1). The inhibition in corpora lutea was observed significantly high ($P < 0.05$) with liposomal gel (85 ± 9%) as compared to control gel (46 ± 8%). This inhibition was observed only for two days with the control gel, while liposomal gel exhibited inhibition for seven days. This may be attributed to the formation of liposomal depot in the vaginal mucosa and controlled and sustained release of drug from the liposome.

It can be concluded that the liposomal formulation containing progesterone exhibited significant therapeutic potential as demonstrated by in vivo progestational activity. Moreover, a prolonged activity of the drug was achieved due to the formation depot in vaginal mucosa. However, detailed study with defined clinical protocol is required to establish utility of such systems.

ACKNOWLEDGMENTS

The authors are thankful to Unichem Laboratories Ltd., Bombay, India for providing progesterone as gift sample. Thanks are due to the University Grants Commission New Delhi (India) for financial assistance and Head, Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar, India for providing necessary facilities.

REFERENCES

1. J. F. Roche, J. Reprod. Fert., 43, 471(1975).
2. S. Hwang, E. Owada, T. Yotsuyanagi, L. Suhardjia, N. F. H. Ho, G. L. Flynn and W. I. Higuchi, J. Pharm. Sci., 65, 1574(1976).
3. D. P. Benziger and J. Edelson, Drug Metab. Rev., 14, 137(1983).
4. S. Hwang, E. Owada, L. Suhardjia, N. F. H. Ho, G. L. Flynn, and W. I. Higuchi, J. Pharm. Sci., 66, 781(1977).
5. Y. W. Chien, Novel Drug Delivery Systems, Marcel Dekker Inc., New York, 1982, p. 51.
6. F. S. K. Barar, Essential of Pharmacotherapeutics, 2nd edition, S. Chand & Co., 1987, p589.
7. A. D. Bangham, Prog. Biophys. Mol. Biol., 18, 31 (1965).
8. M. Kabadi and Y. W. Chien, Drug Dev. Ind. Pharm., 11, 1313(1985).
9. P. Hebborn, Screening Methods in Pharmacology (Robert A. Turner and P. Hebborn, eds.), Vol. II, Academic Press, New York, 1971, p. 105.
10. R. S. Langer and N. A. Peppas, Biomaterials, 2, 201(1981).