AAPS PharmSciTech, Vol. 9, No. 4, December 2008 (© 2008)
DOI: 10.1208/s12249-008-9144-7

Research Article

Carbopol-Based Gels for Nasal Delivery of Progesterone

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Received 29 July 2008; accepted 24 August 2008; published online 11 October 2008

Abstract. The purpose of this study was to investigate the nasal absorption of progesterone from carbopol-based nasal gels in rabbits. Progesterone nasal gels were prepared by dispersing carbopol 974 (1%, 1.5%, and 2%) in distilled water followed by addition of progesterone/progesterone–β cyclodextrin complex dissolved in propylene glycol then neutralization. The potential use of β cyclodextrin (CD) as nasal absorption enhancer by simple addition, as a physical mixture and as a complex with progesterone was investigated. The absolute bioavailability of progesterone from nasal gels in rabbits was studied by estimating the serum progesterone level by competitive solid-phase enzyme immunoassay in comparison to intravenous injection. The carbopol gel formulations produced a significant increase in bioavailability. CD complex promotes the nasal absorption of progesterone from carbopol gels as compared with gels where the CD is added by simple addition and gels which do not contain CD. This method of addition of CD as an inclusion complex in the gels could be considered as a preferred platform in nasal drug administration.

KEY WORDS: cyclodextrins; gels; nasal delivery; progesterone.

INTRODUCTION

Progesterone is a naturally occurring progestin (1) secreted by the corpus luteum and is a prototype of the progestins and apart from its main progestational action exerts antiandrogenic (2) and anti-mineralcorticoid (3,4) effects. Progesterone is a lipophilic drug used to control reproductive function and as postmenopausal therapy. It is used in the treatment of amenorrhea and for the treatment of abnormal uterine bleeding caused by hormonal imbalance in patients without underlying organic pathology such as fibroids or uterine cancer. Until now, no synthetic progestin is able to mimic the same hormonal activities as the native hormone. However, the oral delivery of progesterone is limited since it is not tolerated in higher doses and its oral bioavailability is poor due to its intense hepatic metabolism. This greatly limits the efficacy of peroral administration. Non-oral forms have been conceived via the nasal, rectal, and vaginal route.

Nasal delivery is one of the most attractive non-invasive routes (5) for drugs because of relatively high permeability of nasal epithelium membrane, avoidance of first pass metabolism and improvement of patient compliance. However the bioavailability achieved following nasal administration is usually very low. Nasal mucociliary clearance is one of the most important limiting factors to nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained drug administration. Different strategies have been explored to improve the absorption of drugs through nasal mucosa including using bioadhesive polymers, chemical penetration enhancers, and proteolytic enzyme inhibitors and designing suitable dosage formulations. Of these approaches, the use of bioadhesive polymers has proven to be effective. Some polymers (mucoadhesive polymers) can adhere onto the nasal mucosa for reasonably prolonged periods, preventing rapid nasal clearance. Their use is therefore an avenue for improving nasal absorption as well as prolonging the duration of action of intranasally administered drugs. Various bioadhesive polymers (6) such as polyacrylic acids (7) (eg. carbopol, polymethyl methacrylate) in gel forms prolong the residence time. Polymer gels and mucoadhesive polymers (8) have been studied for the mucosal delivery of various compounds ranging from small molecules to macromolecular drugs. The use of nasal bioadhesive gels (9) blended with an appropriate chemical permeation enhancer can be used to provide enhanced bioavailability.

Cyclodextrins are a group of cyclic oligosaccharides obtained from enzymatic degradation of starch. Cyclodextrins (10) have been widely used to improve the delivery of drugs by nasal administration. Improved nasal delivery has been attributed to changes in nasal mucosa permeability, alterations in drug solubility and in case of some prodrugs and peptides, a change in the metabolism rate of the drugs at the site of delivery. Literature (11) has shown that CD does not cause any detrimental toxicological effect on the nasal mucosa. The objective of this study was to develop nasal gel containing progesterone as the model drug. To achieve the objective, independent formulation variables such as total

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percentage of polymer content and method of addition of cyclodextrin were examined.

MATERIALS AND METHODS

Progesterone was a gift sample from AIN Medicare (I), Chennai. Carpobol was a kind gift from Noveon Inc., Mumbai. Other materials purchased from commercial sources were: β cyclodextrin (S.D. Fine Chemicals), triethanolamine (S.D. Fine Chemicals), propylene glycol (Lab Chemicals).

Preparation of Progesterone–β Cyclodextrin Inclusion Complex

Progesterone–CD (12,13) inclusion complex was prepared by freeze drying technique. CD was first dissolved in distilled water. Progesterone dissolved in ethanol was added to this solution in carefully predetermined molar ratio of 1:1. The entire solution was stirred with a magnetic stirrer for 7 days at room temperature. The suspension was filtered through 0.22-m filter. The filtrate was frozen at −40°C for 10 h in a Telstar L-3 freeze drier and then a vacuum was applied to obtain a pressure of 0.05 mbar. Temperature was increased from −40°C to 0°C in a 37 h cycle and after 2 h at this temperature it was increased to 30°C over 6 h. The prepared complex was characterized by IR and DSC studies.

Fourier Transform Spectroscopy. Infrared (IR) spectra (14) of the freeze-dried inclusion complex and pure drug were recorded with a Fourier transform spectroscopy (FTIR) spectrophotometer. The samples were prepared by processing compressed KBr disks.

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) analysis was performed using a Perkin Elmer DSC 6. All the samples (5–10 mg) were heated in crimped aluminum pans at a scanning rate of 10°C/min in the temperature range 25°C to 200°C

Preparation of Gels

Carbopol 974 (15) was delivered as dry flocculated powders consisting of primary particles with an average diameter of about 0.2 μm. Each primary particle contains a network of cross-linked polymer chains and will swell up to 1,000 times their volume in contact with water to form a continuous gel when exposed to a suitable pH.

The composition for the various formulations is shown in Table I. Carbopol was used as the mucoadhesive polymer. Propylene glycol was used as the solubilizer for the drug. Total quantity of 10 g of formulation was prepared for each batch.

In case of formulations F1, F4, and F7, progesterone was dissolved in propylene glycol and sonicated (Vibra Cell VCX 500, Sonics & Materials) for 5 min. Required quantity of carbopol was wet blended thoroughly. This was then suspended in water and allowed to swell. The polymeric dispersions were stirred in a magnetic stirrer for 60 min to which the drug solution was incorporated in a drop-wise manner and the pH was then adjusted to 7.0 by adding triethanolamine.

In case of formulations F2, F5, and F8, carbopol was added to water in which the required amount of cyclodextrin were dispersed by sonication (Vibra Cell VCX 500, Sonics & Materials) for 30 min. The polymeric dispersions were stirred in a magnetic stirrer for 60 min to which the drug solution (prepared as mentioned above) was incorporated in a drop-wise manner and the pH was then adjusted to 7.0 by adding triethanolamine.

In case of formulations F3, F6, and F9, carbopol was added to water in which the required amount of progesterone–CD inclusion complex was dispersed by sonication (Vibra Cell VCX 500, Sonics & Materials) for 30 min. These polymeric dispersions were stirred in a magnetic stirrer for 60 min and the pH was then adjusted to 7.0 by adding triethanolamine.

Ex vivo permeation studies. The *ex vivo* permeation study was carried out following the procedure described by Steffen Lang *et al.* (16). Tissue with nasal mucosa was excised from the noses of freshly slaughtered sheep. After removing the skin, tissue containing nasal mucosa was cut off with a sharp knife from the frontal part of the nasal conch (conchae nasals dorsales) above the os incisivum starting from the incisura nasoincisiva. The excised tissue was stored on ice during transport to the laboratory. At no more than 30 min after the excision, the mucosa was separated from the underlying cartilage by blunt stripping using a pair of tweezers. Samples were taken and inserted into the diffusion chambers, the apical side of the tissue typically facing the donor compartment. The gel equivalent to 0.5 mg of progesterone was place on the upper side of the nasal mucosa. The donor and the receiver compartment containing 20 ml of pH 6.2 phosphate buffer were kept in intimate contact and the temperature was maintained at 32°C. The whole assembly was kept on a magnetic stirrer and stirred continuously. The samples were withdrawn at definite time intervals and equal amount of the phosphate buffer was replaced. The transport rate k was determined from the slope obtained by linear regression of the fraction of the drug absorbed as a function

Table I. Composition of Gels

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Progesterone (mg)	10	10	–	10	10	–	10	10	–
CD-P complex equivalent to progesterone (mg)	–	–	10	–	–	10	–	–	10
β-CD (mg)	–	181	–	–	181	–	–	181	–
Propylene glycol (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 974 (mg)	100	100	100	100	100	100	100	100	100
Water q.s (g)	10	10	10	10	10	10	10	10	10

Table II. Kinetic Values and Release Profile of Gels

Formulation	*k* value	Release at 2 h (%)
F1	$0.320\times10^{-2}\pm1.17$	30.31±0.72
F2	$0.591\times10^{-2}\pm2.18$	62.13±1.50
F3	$0.906\times10^{-2}\pm2.33$	91.52±0.72
F4	$0.313\times10^{-2}\pm0.17$	34.47±3.87
F5	$0.542\times10^{-2}\pm0.18$	56.53±4.26
F6	$0.839\times10^{-2}\pm1.34$	82.83±1.36
F7	$0.306\times10^{-2}\pm3.11$	31.8±0.36
F8	$0.501\times10^{-2}\pm2.32$	50.73±1.23
F9	$0.824\times10^{-2}\pm0.33$	81.87±12.7

of time and the results are given in Table II and the release profile represented in Figs. 3, 4 and 5.

In Vivo studies. Rabbits (17) with a mean weight of 3± 0.5 kg were acclimatized for 2 weeks before the study. The animals were fasted overnight prior to drug administration with free access to water. A 1-week wash-out period was allowed between experiments. The formulations were administered intranasally at a dose of 40 μg/kg body weight into each nostril. The animals were kept conscious during the experiments and permitted to breathe normally through the nostrils. One group of rabbits received an intravenous injection of parenteral solution (40 μg/kg of progesterone) to calculate the absolute bioavailability. Blood samples were withdrawn from the marginal ear vein at 10, 30, 45, 60, 90, 120, 180 240, and 300 min after administration. Plasma was separated by centrifugation at 2,000 rpm and progesterone drug content was determined by using a competitive solid phase enzyme immunoassay (Progesterone EIA, Biomerieux Italia, I-Rome)

The pharmacokinetic parameters area under the concentration-time curve from 0 to 300 min (AUC), peak concentration (C_{max}) and time to reach peak concentration (t_{max}) after both intravenous and intranasal administration were calculated independently of kinetic models by trapezoidal rule and from raw data and shown in Table III. The absolute bioavailability following intranasal administration was determined by dividing the intranasal AUC by the intravenous AUC.

Statistics. All data are presented as arithmetic mean values±SD.

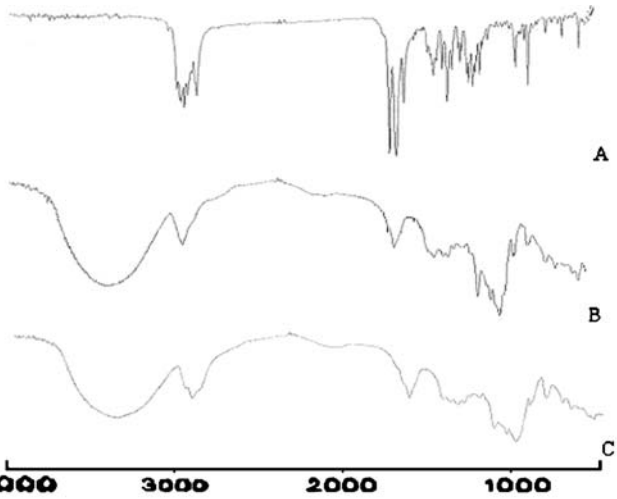

Fig. 1. Infra-Red spectra: **a** Progesterone, **b** β-CD and **c** Progesterone-β-CD complex

RESULTS AND DISCUSSION

FTIR. Figure 1 shows the IR spectra of progesterone and progesterone–cyclodextrin complex. The peak at 1,661 and 1,698 cm^{-1} are assigned to carbonyl stretching bands of C_3 and C_{20} in progesterone respectively. In the spectrum of the complex, the stretching bands of carbonyl group disappeared. These spectral changes can be explained by the dissociation of the intermolecular hydrogen bonds of progesterone through inclusion complexation.

DSC. DSC was performed on the raw materials and on the inclusion complex (Fig. 2). The thermogram of progesterone shows the characteristic endothermic peak at 136°C corresponding to drug melting. The thermal behavior of the other components used for the complex formation, i.e. CD shows no phenomena in the same temperature interval. The freeze dried progesterone–CD showed a disappearance of the endothermal peak of progesterone, indicating that the drug permeates into the CD cavity replacing the water molecules confirming drug amorphization and/or inclusion complex formation.

Table III. Pharmacodynamic Parameters of Nasal Gels

Formulation	$AUC_{(0-\infty)}$ ng.min/ml	C_{max} ng/ml	T_{max} min	Bioavailability (%)
i.v.	27891±2.5	25.1±2.3	10	–
F1	11158±7.8	12.1±3.6	10	40.98±4.7
F2	17146±11.9	15.4±4.2	10	62.47±9.3
F3	22341±8.6	20.4±5.3	10	80.1±14.3
F4	11002±10.2	11.6±2.3	10	39.4±12.1
F5	15428±3.6	14.6±6.3	10	55.3±5.6
F6	21201±4.9	18.7±4.6	10	76.0±8.4
F7	9854±13.6	11.2±5.3	10	35.3±5.3
F8	13251±9.7	13.6±2.1	10	47.5±8.6
F9	19803±11.4	18.2±1.7	10	71.0±10.2

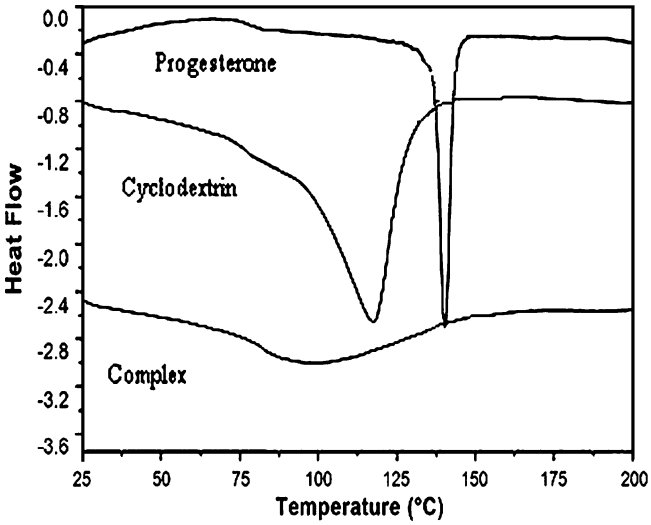

Fig. 2. DSC curves of progesterone, β-CD and complex

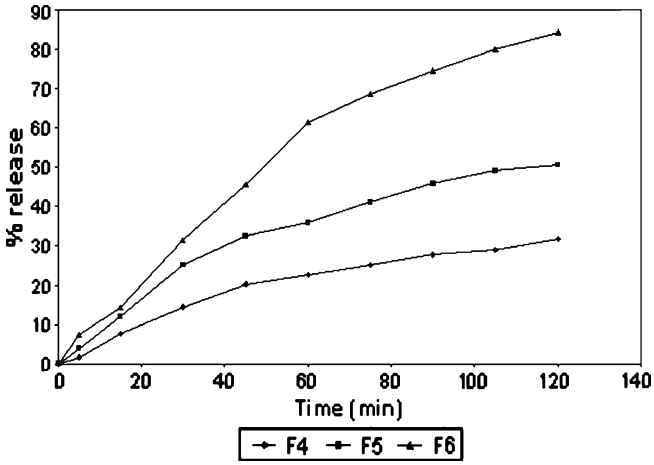

Fig. 4. Drug release curves of progesterone form gels F4, F5, and F6

Release studies. The release profile and *k* value of the various gels are shown in Figs. 3, 4 and 5 and Table II. The rate of release of progesterone from gels with no cyclodextrin was the slowest for all concentrations of the polymer while that with progesterone as a cyclodextrin complex were the fastest. The polymer concentration also affected the release but this effect was found to be minimum as compared to the method of addition of progesterone. The polymer concentration, however, can seldom be used as an effective tool for tailoring the release rate since the formulation should have good rheological properties in order to have long residence time at the site of drug absorption.

CDs have the potential to enhance drug release from polymeric systems by increasing the concentration of diffusible species within the matrix (18). The addition of cyclodextrin to polymeric systems enhances drug release by its ability to enhance aqueous solubility of the drug, while concomitantly acting as a water-leachable component and promoting matrix component.

Cumulative drug *in vitro* release from gels (1%) containing 1:1 drug–β-CD ratio was 91.9% after 2 h which was more as compared with 34.2% for gel-containing drug alone. Incase of 1.5% gels the % release of drug was 32%, 56%, and 88% for formulations containing progesterone alone, as a mixture with CD and complex respectively. Similarly also the same case is seen in case of gels with 2% carbopol. The rate of release from 2% carbopol gels was the slowest while that from 1% carbopol gels were the fastest. The carbopol concentration affected the release to a small extent. It is seen that the carbopol concentration is not the determining factor in the enhancement of drug release but the method of addition of CD plays a vital role.

In vivo studies. The changes in bioavailability of progesterone following administration of different formulations are summarized in Table III. The absolute bioavailability of F3 was 80.1±14.3 whereas the bioavailability of the F1 was 40.9±4.7. Thus about a twofold increase in bioavailability was obtained using progesterone as a complex with CD. Carbopols function as absorption enhancers in two ways. First, they adhere to the mucosal surface, thereby increasing the contact time of the drug with the mucosa and second they induce a transient opening of epithelial cell tight junction. On the other hand the enhanced release of drug

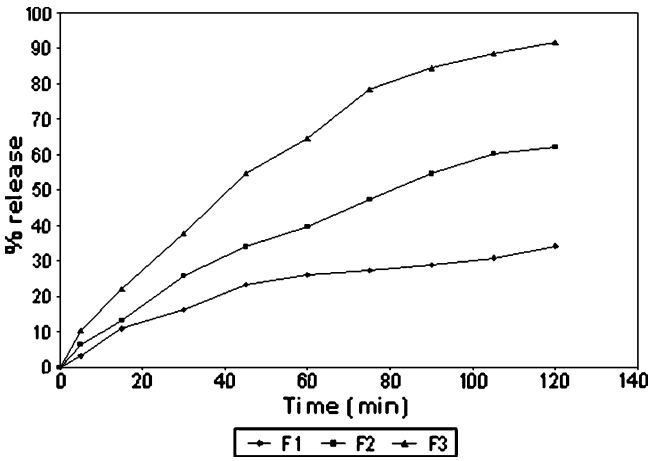

Fig. 3. Drug release curves of progesterone form gels F1, F2, and F3

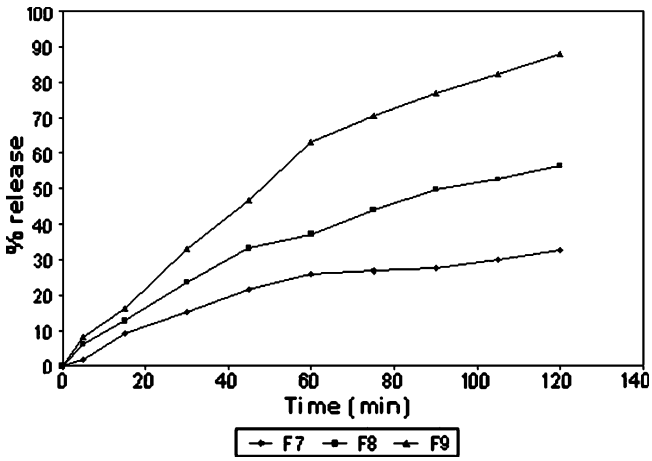

Fig. 5. Drug release curves of progesterone from gels F7, F8, and F9

was a result of the incorporated cyclodextrin dissolving upon contact with water, increasing the porosity of the matrix and also allowing the removal of the drug via its inclusion within the CD cavity. This result is similar to what was observed in previous studies of Guo *et al.* (19) and Samy *et al.* (20).

CONCLUSION

To obtain the therapeutically significant plasma levels of progesterone following nasal application it is necessary to employ a system that will promote absorption. The results from the present study indicate that it is possible to achieve enhanced absorption of progesterone using carbopol nasal gel that could be improved using CD as the absorption enhancer and altering the method of addition.

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