RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2007.33.057

ScienceAsia 33 (2007): **57-60**

Development of Oxytocin Nasal Gel using Natural Mucoadhesive Agent obtained from the Fruits of *Dellinia indica.* L.

Ketousetuo Kuotsu and Amal Kumar Bandyopadhyay*

Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India.

* Corresponding author, E-mail: akbju@yahoo.com

Received 16 Dec 2005
Accepted 27 Jul 2006

ABSTRACT: A new nasal gel formulation has been developed using a natural mucoadhesive agent obtained from the fruit of *Dellinia indica* L. The mucoadhesive strength and viscosity of this natural mucoadhesive agent was found to be higher in comparison to the synthetic polymers, namely hydroxy propyl methyl cellulose (HPMC) and carbopol 934, which are conventionally used for a similar purpose. *In vitro* drug release characteristics using a Franz-diffusion cell and excised bovine nasal membrane was also found to be better in comparison to the above synthetic polymers. This patient friendly, needle free dosage form may replace the oxytocin injections in the future.

KEYWORDS: Oxytocin; *dellinia indica* L., mucoadhesive, nasal drug delivery, carbopol 934, hydroxypropyl methyl cellulose.

INTRODUCTION

Due to the advancement of biotechnology and genetic engineering, many new drugs are being developed which are small proteins or peptides. To deliver these drugs through non-parenteral routes is currently a challenging research area, since injection is one of the most hazardous routes of drug delivery. Administration of these drugs through nasal routes may be a very good alternative to injection.

Nasal transport can be studied in *in vitro* models using explanted nasal tissue such as porcine or bovine mucosa transferred to an "Ussing chamber", or cell culture models of nasal cells. *In-situ* perfusion of the nasal mucosa in rats or pharmacokinetic studies in animals has contributed much to the knowledge of nasal bioavailabilities, but studies elucidating transport mechanisms are difficult to perform[1-3]. *In vitro* permeability studies offer advantages over *in vivo* studies in that they can be performed more rapidly, involve fewer animals and simpler analytical procedures can be followed, since the presence of plasma proteins in the samples is avoided. Additionally, since pre- and post-mucosal factors are eliminated with *in vitro* techniques, the systems are more standardized[4, 5].

The aim of this study was to develop a nasal delivery system of oxytocin, which is used in the form of injections only, and to evaluate the drug release pattern in an *in vitro* system. The drug delivery system was developed using a natural mucoadhesive agent extracted from *dellinia* fruits.

MATERIALS AND METHODS

Materials

Oxytocin powder was obtained as a gift sample from Hemmo Pharma, Mumbai. *Dellinia indica* fruits were purchased from local vendors. Hydroxy propyl methyl cellulose (HPMC) 5cPs and carbopol 934 were purchased from s. d. fine-chem. Ltd., Mumbai, India. HPLC solvents were purchased from Merck Ltd., Mumbai, India. All other reagents and chemicals used were of analytical grade.

Methods

Extraction of Mucoadhesive Agents from the Fruits of *D. Indica* L.

Dellinia fruit mucilage was extracted following the methods of Rao et al.[6, 7] with little modifications. 500 g of *dellinia* fruit was soaked in double distilled water and boiled under stirring condition in a water bath until a thick slurry was produced. This solution was cooled and kept in the refrigerator overnight so that the undissolved portion settles. The upper clear solution was decanted and centrifuged at 500 rpm for 20 min. The supernatant was separated, concentrated at 60 ^{o}C on a water bath until the volume was reduced to one fourth of its original volume, and cooled to room temperature. The concentrate was poured into thrice the volume of acetone with constant stirring. The

precipitate was washed repeatedly with acetone, collected, and dried at 50°–60°C under vacuum for 12 hours. The dried material was powdered, passed through 80-mesh screen and stored in a desiccator until used.

Determination of pH

The pH of a 1% w/v solution of the *dellinia* fruit extract (*DFE*), HPMC and Carbopol 934 was measured using a Toshcon pH meter.

Measurement of Viscosity

Viscosity measurements were carried out at1%w/v concentration of *DFE*; HPMC and Carbopol 934 using Toki Sangyo Viscometer TV-10.

Assessment of Mucoadhesive Properties

The mucoadhesive property of *DFE* was determined by the Shear stress method[8] and the Park and Robinson method[9], and the results were compared with synthetic polymers. In this study, 0.5% w/v and 1% w/v solutions of the polymers were used for the shear stress method and the Park and Robinson method respectively.

Preparation of the Nasal Gel

Nasal gels were prepared using natural and synthetic mucoadhesive polymers at its optimum concentrations as determined by viscometric studies. The materials were dissolved in a measured volume of nasal solution (0.65% NaCl, 0.04% KH_2PO_4, ph 6.2, 0.09% K_2HPO_4 and 0.02% benzalkonium chloride). The contents were sonicated using Pci Ultrasonic cleaner for 10 min and stirred in a magnetic stirrer for 15 min.

The whole content was sealed and stored in the refrigerator overnight to allow complete swelling. An aliquot amount of oxytocin (IU) was added and stirred again for 15 min. The prepared gel was sonicated to ensure the complete removal of air bubbles. Similarly gels were prepared using different enhancers.

Studies on the Release Pattern of Oxytocin

Preparation of Excised Bovine Nasal Mucosa

Bovine nasal mucosa was obtained from the local slaughterhouse. After removing the skin, the nose was stored on ice in Kreb's buffer solution during transport to the laboratory. The septum wall was fully exposed by a longitudinal incision through the lateral wall of the nose. The septum mucosa was carefully removed from the underlying bone by cutting along the whole septum and pulling the mucosa off the septum with homeostatic forceps. The cavity mucosa was also carefully removed from the conchae and lower cavity using homeostatic forceps after exposing the cavity each side of the septum. The mucosal tissues were then immediately immersed in Ringer's Solution[10].

Studies on the Release of the Drug from the Gel

The Franz diffusion cell[11] was used for the drug release study. The diffusion chamber with an exposed tissue surface area of 2.54 cm^2 was filled with 100 ml phosphate buffer (31.5 mM, pH 7.0). The excised nasal mucosal membrane was secured over the mouth of the upper tube keeping the mucus side exposed to the gel. For equilibration, the mucosae were preincubated with preheated buffer for ~30 min. Oxytocin containing gel (1 mg/ml) placed on the membrane was dispersed in 100 ml phosphate buffer (31.5 mM, pH 7.0), and stirred at a constant rate by a PTFE-coated magnetic bar at 600 min^{-1}. Cells were kept under constant oxycarbon flow (95% O_2, 5% CO_2). Throughout the studies, the buffer solution in the chamber was maintained at 37 °C connecting the Franz diffusion cell with the water bath. At predetermined time, 1 ml of sample was taken, and simultaneously replaced with the same volume of prewarmed (37°C) fresh buffer solution. Samples so collected were diluted to a suitable concentration and were analyzed by HPLC method as described by Li et al[12].

The mobile phase used 20% acetonitrile in 31.5 mM phosphate buffer at pH 7, at a flow rate of 1.5 ml/min, and the HPLC system was equipped with a Jasco PU 1580 unit pump, a microliter # 702, Hamilton injector, a Thermo Quest Hypersil C8 (4.6 mm x 150 mm) column and a Model Jasco UV1575 detector. The estimation was done at 215 nm. Chromatographic peaks were automatically integrated and recorded by Data Apex Chromatographic Station for Windows 17 data module (Data Apex; Prague, Czech Republic).

Evaluation of the Effect of Enhancers on Drug Release

Enhancers, such as sodium taurocholate, microcrystalline cellulose and sodium thioglycollate, were used at 0.5% concentration to evaluate the effect of these materials on drug release characteristics from natural and synthetic nasal gels[13].

Results and Discussions

Determination of pH

The pH of *DFE* was in the range of 5.8 to 6.8, whereas the pH of HPMC and carbopol 934 were 6.2 and 3.5, respectively. As the pH of nasal mucosa varies between 5.5 and 6.5, DFE was found to be suitable for preparing nasal gels.

Measurement of Viscosities

Results of the viscosity measurements of 1% w/v solutions of *DFE*, HPMC and Carbopol are shown in Fig 1. The viscosity of *DFE* ranges from 9-13 cPs while that of HPMC and Carbopol 934 was 1.3-2.8 cPs and 6.4-

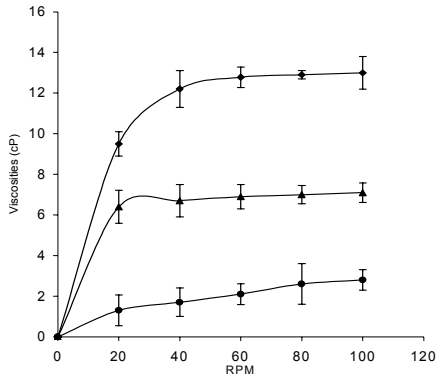


Fig 1. Viscosity of the 1% w/v solutions of the natural mucoadhesive extract *DFE* and the synthetic polymers HPMC and carbopol 934 . Experimental temperature was maintained at 37±1 °C. Values are expressed as the mean of 6 observations.

7.1 cPs, respectively. Thus the viscosity of the *DFE* was more than that of the synthetic polymers.

Assessment of Mucoadhesive Properties

The results of the mucoadhesive property were recorded for various polymers with different contact time (Fig 2 & 3). It is observed that increased contact time increased the adhesion strength allowing for more adhesion[14]. Probably increasing contact time might have reduced the hydration due to evaporation facilitating higher adhesion[15].

The results showed that *DFE*, having high molecular weight and high viscosity, exhibited higher adhesion and better mucoadhesive property in comparison to the synthetic polymers (HPMC and carbopol 934) at the same concentration. This may be due to the presence

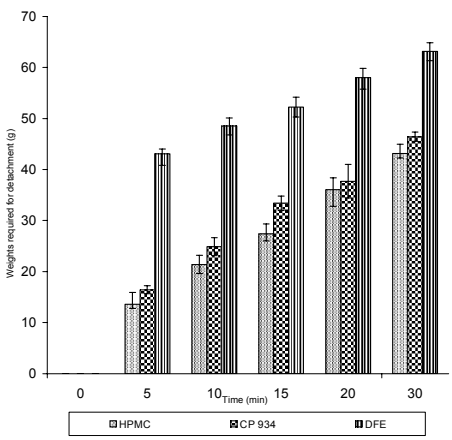


Fig 2. Bioadhesive property of the 0.5% w/v solutions of the natural mucoadhesive extract *DFE* and the synthetic polymers HPMC and carbopol 934 as determined by shear stress method. Experimental temperature was maintained at 37±1 °C. Values are expressed as the mean of 6 observations.

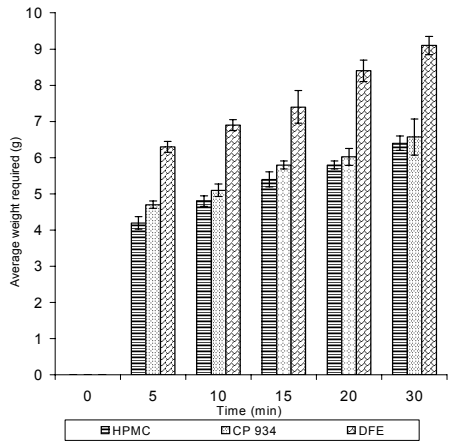


Fig 3. Comparative result of mucoadhesive property of the natural mucoadhesive extract (DFE) with synthetic polymers HPMC and carbopol 934 by Park & Robinson method. All the polymers used in 1% w/v solution. Experimental temperature was maintained at 37°C±1. Values are expressed as the mean of 6 observations.

of numerous disulphide bridges and carboxyl and hydroxyl groups, which adopt more favorable macromolecular conformation, and accessibility of its hydrogen-binding groups, when compared with other polymers. HPMC, being a cellulose derivative, formed weaker bonds with mucus, which may be due to either a decrease in available hydrogen binding sites or unfavorable entanglement with the mucus.

In vitro Dissolution Studies of Oxytocin with DFE and the Synthetic Polymers

In case of *DFE*, 100% drug was released within 3.2 hours, whereas for the synthetic polymers (HPMC and carbopol 934), the oxytocin was released after 5 hours (Fig. 4).

Effect of the Enhancers

Comparative evaluations of different enhancers on

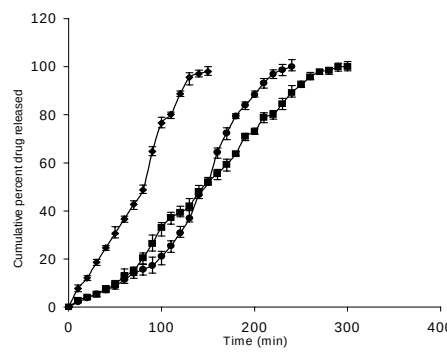
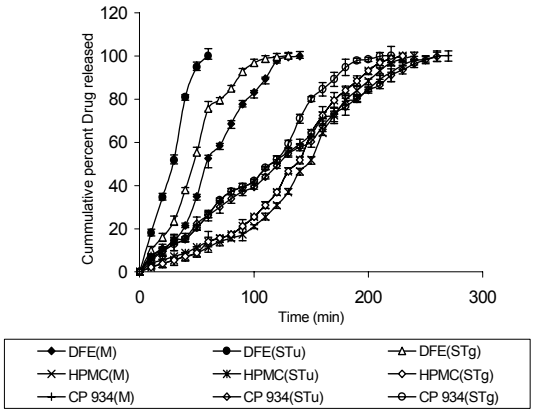


Fig 4. Cumulative percentage of release of oxytocin from the natural mucoadhesive extract *DFE* and the synthetic polymers HPMC and carbopol 934 using bovine nasal membrane. Values are expressed as the mean of 6 observations.

the prepared nasal formulation are given in Fig 5. In the absence of any enhancer, 100% oxytocin was released within 3 hours and15 minutes from the *DFE* nasal gel. However, when sodium taurocholate was used as an enhancer, 100% drug was released within 55 minutes. In the case of carbopol 934 and HPMC, 100% of the drug was released in about 6 hours when no enhancer was used. However, with sodium taurocholate, 100% of the drug was released in 5 hours for carbopol 934. Not much change was noticed in the case of HPMC.



M- Microcrystalline cellulose.
STu- Sodium tourocholate.
STg – Sodium thioglycollate.

Fig 5. The effect of different enhancers on cumulative percentage of release of oxytocin from the natural mucoadhesive extract *DFE* and the synthetic polymers HPMC and carbopol 934 using bovine nasal membrane. Values are expressed as the mean of 6 observations.

Conclusion

It can be concluded from the present study that *dellinia* fruit extract mucilage is a better mucoadhesive agent than HPMC and carbopol 934 with respect to mucoadhesive strength, gelling properties, and drug release. Permeation studies through bovine nasal mucosa suggest that sodium taurocholate is a better permeation enhancer for the release of oxytocin from *dellinia* fruit mucilage based nasal gel than other enhancers used in the study. Since this natural mucoadhesive agent is edible, it is easily biodegradable and non-allergic. Oxytocin is routinely administered parenterally. Since injection is one of the most hazardous routes of drug administration and has the least patient compliance, this nasal delivery system may provide an alternative to the conventional oxytocin injection. This work will definitely add a new dimension in the area of novel drug delivery research.

Acknowledgements

Authors are grateful to University Grants Commission, Bahadurshah Zafar Marg, New Delhi, India, for providing all the financial assistance for this project.

References

1. Nakamura K., Maitani Y., Lowman A.M., Takayama K., Peppas N.A. and Nagai T., (1999) Uptake and release of budesonide from mucoadhesive, pH sensitive copolymers and their application to nasal delivery. *J. Control. Rel.* **61**, 329-35.
2. Oechslein C., (1994) Particulate carrier systems for the enhancement of nasal drug absorption, in: PhD Thesis Marburg. 23-50.
3. Lee C.P., de Vrueh R.L.A. and Smith P.L. (1997) Selection of development candidates based on in vitro permeability measurements. Adv. *Drug Del. Rev.* **23**, 47– 62.
4. Jorgensen L. and Bechgaard E., (1994) Intranasal permeation of thyrotropin releasing hormone: in vitro study of permeation and enzymatic degradation. *Int. J. Pharm.* **107**, 231–7.
5. Kubo H., Hosoya K.I., Natsume H., Sugibayashi K. and Morimoto Y., (1994) In vitro permeation of several model drugs across rabbit nasal mucosa. *Int. J. Pharm.* **103**, 27–36.
6. Khullar P., Khar R.K. and Agarwal S.P., (1998) Evaluation of guar gum in the preparation of sustained-release matrix tablets. *Drug. Dev. Ind. Pharm.* **24**,1095-9.
7. Rao P.S., and Srivastava H.C., (1973) Tamarind in Whistler RL (ed), Industrial Gums. 2nd Ed. New York; Academic Press, 369-411.
8. Rao Y.M., Vani G. and Bala Ramesha Chary R., (1998) Design and evaluation of mucoadhesive drug delivery systems. *Ind. Drugs*. **35**,558-65.
9. Park K & Robinson R, (1984) Bioadhesive platforms for oral-controlled drug delivery: method to study bioadhesion, *Int. J. Pharm*, **19**,107-27.
10. Schmidt M.C., Simmen D., Hilbe M., Boderke P., Ditzinger G.N., Sandow J.R., Lang S., Rubas W. and Merkle H.P., (2000) Validation of Excised Bovine Nasal Mucosa as In Vitro Model to Study Drug Transport and Metabolic Pathways in Nasal Epithelium. *J. Pharm. Sci*. **89**,396-407.
11. Frantz S.W., (1990) Instrumentation and methodology for in vitro skin diffusion cells in methodology for skin absorption. In: Methods for Skin Absorption (Kemppainen B.W.,Reifenrath W.G., Eds), CRC Press, Florida, 35-59.
12. Li C., Bhatt P.P., Johnston T.P., (1997) Transmucosal delivey of oxytocin to rabbits using mucoadhesive buccal patch. *Pharm. Dev. Tech*. **2(3)**, 256-74.
13. Wheatley M.A., Dent J., Wheeldon E.B. and Smith P.L., (1988) Nasal drug delivery: an in vitro characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancers. *J.Control. Rel.* **8**,167–77.
14. Gandhi R.B., Robinson J.R., (1988) Bioadhesion in Drug Delivery. *Ind. J. Pharm. Sci.* **50**,145-52.
15. Ahuja A., Khar R.K. and Ali J. (1997) Mucoadhesive Drug Delivery Systems. *Drug Dev. Ind. Pharm.* **23**,489-515.