Formulation and evaluation of insulin enteric microspheres for oral drug delivery

Santosh Kumar Jindal[1*], Malkeet Singh[2], Manish Goswami[1]

[1]Akal College of Pharmacy and Technical Education, Mastuana, Sangrur – 148001 (Punjab) INDIA
[2]Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala - 147002 (Punjab) INDIA

Abstract

The goal of the present study was to formulate oral drug delivery device for insulin and to protect the sensitive drug from digestive enzymes and proteolytic degradation in stomach and upper part of gastro intestinal tract (GIT). So, for this purpose insulin enteric microspheres (EMS) were prepared using Hydroxy propyl methyl cellulose acetate succinate as enteric polymer, Bacitracin as protease inhibitor and Sodium oleate as absorption enhancer. In-vitro drug release studies determined that almost no drug was released in HCl (pH 1.2) for 2 hours and then maximum amount of drug was released within 70 minutes in Phosphate buffer (pH 7.4). In-vivo studies on male wistar rats confirmed a remarkable decrease in blood glucose level after 2 hours of administration of insulin EMS.

Key words: Insulin, insulin oral drug delivery, hydroxy propyl methyl cellulose acetate succinate, enteric microspheres, protease inhibitor, absorption enhancer.

Introduction

Since the discovery of insulin in 1921, it is generally administered parenterally via sub cutaneous route for the treatment of insulin dependent diabetes mellitus (IDDM) or type-1 diabetes. Subcutaneous route which is most commonly used clinically now a day, but this clinical therapy suffers from many disadvantages e.g. Local discomfort, inconvenience of multiple administration and occasional hyperinsulinemia due to overdose (DeVries et al. 2002, Herman et al. 2005, Pickup et al. 2002). So, oral route is considered to be more convenient and most acceptable route of drug administration for chronic therapy. However the insulin delivered by this route in not as effective as by sub cutaneous route because of poor absorption in gastro intestinal tract and degradation by gastric acid or proteolytic enzymes which are most important barriers limiting the absorption of insulin (Wu and Robinson 1991, Carino and Mathowitz 1993, Modi et al. 2002, Marschutz et al. 2000, Brenkop-Schnurch and Thaler 2000, Hosny et al. 2006, Krauland et al. 2004, Marschutz and Brenkop-Schnurch 2000, Marschutz et al 2000, Mathiowitz et al. 1997). Encapsulation of insulin within EMS could protect the insulin from digestive enzymes in stomach and upper part of GIT, releasing the insulin further down in digestive tract. So, Hydroxy propyl methyl cellulose acetate succinate was used as enteric polymer in this study (Morishita et al. 1993). EMS was formed by $O_1:O_2$ emulsion solvent evaporation method. The processed EMS was having high loading efficiency for drug. EMS of Hydroxy propyl methyl cellulose acetate succinate shows more flexible molecular structure and better solubility in the intestinal solution than others.

---

*Corresponding author: jindalsantosh77@rediffmail.com

Bile salts, Sodium oleate, Sodium glycocholate, Cyclodextrins, Capric acid, Glycyrrhizic acid, Sodium tauro-24,25-dihydrofusidate etc. are known to increase insulin bioavailability through mechanism of enhancing the absorption of insulin across GIT (Donnelly et al. 1998, Morimato et al. 1998, Sakai et al. 1998, Scott-Moncrieff et al. 1994, Yamamoto et al. 1994 I). In the present study, sodium oleate was used as absorption enhancer. Trysin and α- chymotrypsin has been found to be primary proteolytic enzymes responsible for initial cleavage and unfolding of insulin globular structure (Shiling and Mitra 1991). So, insulin must be protected from proteolytic enzymes to maintain its greatest amount intact (Yamamoto et al. 1994 a). For this purpose, a variety of protease inhibitors can be used like Aprotonin, Bacitracin, soybean trypsin inhibitor etc. (Yamamoto et al. 1994 a, Yamamoto et al. 1994 b, Bai and Chang 1996, Schipper et al. 1999, Shao et al. 1994, Morimato et al. 1998, Sakai et al. 1998, Donnelly et al. 1998, Kotze et al. 1999, Martin et al. 1998). In the present study, bacitracin was used as protease inhibitor.

So, the present study was aimed at encapsulation of insulin within enteric microspheres, so as to protect it from digestive enzymes of the stomach and upper part of GIT before it can be absorbed from the lower parts of intestine. Additionally, protease inhibitor and absorption enhancer were used during formulation of EMS to increase the bioavailability of insulin.

Materials and Methods

Materials

Insulin was obtained as gift sample from Torrent Pharmaceuticals Ltd., Indrad (Mehsana) India. Hydroxy propyl methyl cellulose acetate succinate was purchased from Shin-Etsu Chemical Co., Ltd., Branch – Bombay, India. Bacitracin and Sodium oleate were purchased from Sigma Aldrich, Branch – Bangalore, India. All other chemicals used were of analytical grade.

Method

O_1/O_2 emulsion solvent evaporation method was used for preparing insulin EMS (Lin et al. 1991). 20 mg of insulin was dissolved in 5 ml of 90% ethyl alcohol containing 180 mg of Hydroxy propyl methyl cellulose acetate succinate. The solution as the internal oil phase was emulsified with 50 ml liquid paraffin containing 0.5-2% span 80, bacitracin and sodium oleate under homogenization. This O_1/O_2 emulsion was stirred at 300 rpm for 4 hours at room temperature to remove solvents and allow formation of EMS. The EMS were collected via filtration, washed three times with n-hexane and dried under vacuum over night.

Insulin loading and entrapment efficacy

In 2 mg EMS, 1ml of 0.5 M NaOH and 4 ml of 4% bovine serum were added and analyzed by radio immunoassay using coat-A-count insulin kit. Resulting free concentration is subtracted from theoretical concentration (free plus encapsulated) to estimate the drug loading.

Effect of EMS on insulin protection from pepsin

EMS corresponding to 0.5 mg of insulin were weighed precisely in glass vials and incubated with 1ml HCl (0.1mol/l) containing pepsin (18μg) at 37^0C while shaken horizontally at 60-700 rpm. Three vials of EMS were taken out at predetermined time point and were put into an ice water bath; the reaction was further terminated by adding 4ml phosphate buffer solution (pH 7.4). The suspension was shaken to dissolve EMS. Then from resultant solution, 1 ml quantity was taken out and an equivalent volume of the cyclohexane was added to precipitate polymer. The suspension was centrifuged at 3000 rpm for 10 minutes and supernatant solution was analyzed. Same procedure was repeated for an equivalent amount of insulin.

In-vitro release from EMS

EMS corresponding to 1 mg insulin were precisely weighed in glass vials and suspended in 10 ml HCl (pH 1.2) and shaken at 60-70 rpm at 37^{0}C. After 2 hours the suspension of EMS was centrifuged at 3000 rpm for 10 minutes and supernatant was taken for further analysis. EMS were washed with purified water and centrifuged, supernatant was discarded and resulting EMS were suspended in 10 ml of phosphate buffer (pH 7.4) and shaken at 60-70 rpm at 37^{0}C. At predetermined time intervals, 200 μl suspension was taken out as sample while supplementing with the same volume of buffer solution. Then 200 μl cyclohexane was added to the sample to precipitate the enteric polymer. The suspension was centrifuged at 3000 rpm for 10 minutes and concentration of supernatant was analyzed.

In-vivo studies

For in-vivo studies, male wistar rats (200 g) were used. Diabetes was induced in rats by intraperitonial injection of streptozotocin (40 mg/kg of body weight daily for 3 consecutive days) dissolved in citrate buffer at pH 4.5. The rats were considered diabetic when the fastened glucose level exceeds 250 mg/dl at 2 weeks following the streptozotocin treatment.

Prior to administration of insulin-loaded polymer, the animals were fasted for 48 hours. The rats were restrained in supine position. The insulin loaded EMS was administrated via mouth (EMS was loaded into gelatin capsules and then administrated via mouth, gelatin capsules dissolve readily in the stomach). During the experiment 0.2 ml aliquot of blood was collected from the jugular vein at 1, 2, 3, 4, 5, 6, 7 hours following dosing. Blood serum was separated by centrifugation at 300 rpm for 3 hours and stored in freezer until analysis. The serum insulin levels were determined by an enzyme immunoassay using an insulin EIA kit. Serum glucose levels were determined by glucose oxidase method using a glucose- B-test kit.

Analysis of supernatant solution (Plummer., IIIrd Ed.)

Materials

Following materials were used for the analysis of supernatant solution (Plummer., IIIrd Edn.):

1. Alkaline sodium carbonate solution (3 liters): 20 gm/liter Na_2CO_3 in 0.1 mol/liter NaOH.

2. Copper sodium tartrate solution (100 ml): 5g/litre $CuSO_4.5H_2O$ in 10 g/liter sodium, potassium tartrate (Freshly prepared).

3. Alkaline solution (3 liters): Prepared on the day of use by mixing 50 ml of (1) and 1 ml of (2) solutions.

4. Folin-Ciocalteau reagent (500 ml): It is also known as Folin- Phenol reagent. Diluted commercial reagent with an equal volume of water on the day of use. This is solution of sodium tungsten and sodium molybdate in phosphoric acid and hydrochloric acid.

5. Standard protein (1 liter): Albumin solution 0.2 mg/ml.

Procedure

Added 5 ml of alkaline solution to 1 ml of the test solution. Mixed thoroughly and allowed to stand at room temperature for 10 minutes or longer. Added 0.5 ml of diluted Folin-Ciocalteau reagent rapidly with immediate mixing. After 30 minutes, read the extinction against the appropriate blank at 750 nm. Estimated the protein concentration of unknown solution after preparing a standard curve (Plummer., IIIrd Ed.).

Results and Discussion

Insulin loading and entrapment efficiency

The EMS theoretically loaded with 10% (w/w) of insulin. Loading was found to be 8.5% and entrapment was 87%. As a hydrophilic protein, insulin may have diffused from internal aqueous phase to external aqueous phase through the oil phase layer; the incorporated insulin

migrated to external aqueous phase through the porous matrix of microspheres resulting in lowering of drug loading.

Peptic degradation of insulin

The stability of insulin solution and insulin EMS was observed in HCl (0.1mol/l) containing pepsin. Pepsin could digest the insulin within 60 minutes. The same content of insulin in EMS was having better stability to pepsin. The EMS containing the same amount of insulin were incubated in the same content of peptic solution for about 4 hours, the insulin in EMS was digested only to an extent of 21% (Figure 1).
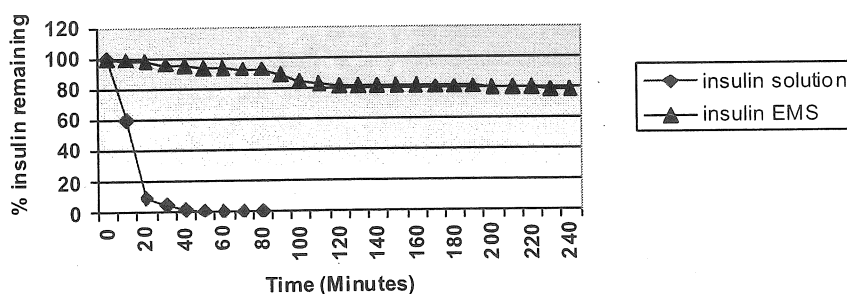

Figure 1. Stability of insulin solution and insulin EMS in peptic solution.

Release of insulin from EMS

The graph shows the insulin release profile from EMS in hydrochloric acid solution (pH 1.2) and phosphate buffer (pH 7.4). In hydrochloric acid solution for 2 hours, almost no release of the drug was observed. Then in phosphate buffer solution, the maximum amount of the drug was released within 70 minutes (Figure 2).
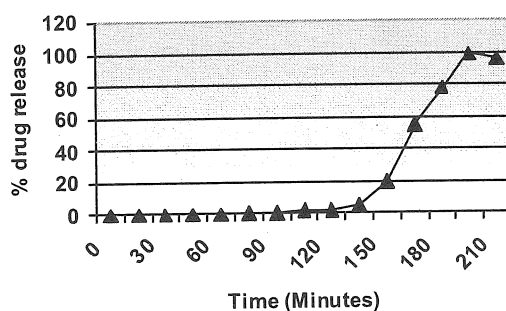

Figure 2. In-vitro release of insulin from EMS.

124

In-vivo hypoglycemic effect of insulin EMS

Observations were made for hypoglycemic effects of insulin oral solution and insulin EMS with absorption enhancer and protease inhibitor (Table 1). EMS with absorption enhancer and protease inhibitor had an intense and lasting hypoglycemic effect. After administration of drug, almost no decrease in blood glucose level was observed for initial 2 hours, but then a remarkable decrease in blood glucose level was observed for more than 5 hours (Figure 3).

Table1. Hypoglycemic effect of insulin oral solution and insulin EMS.

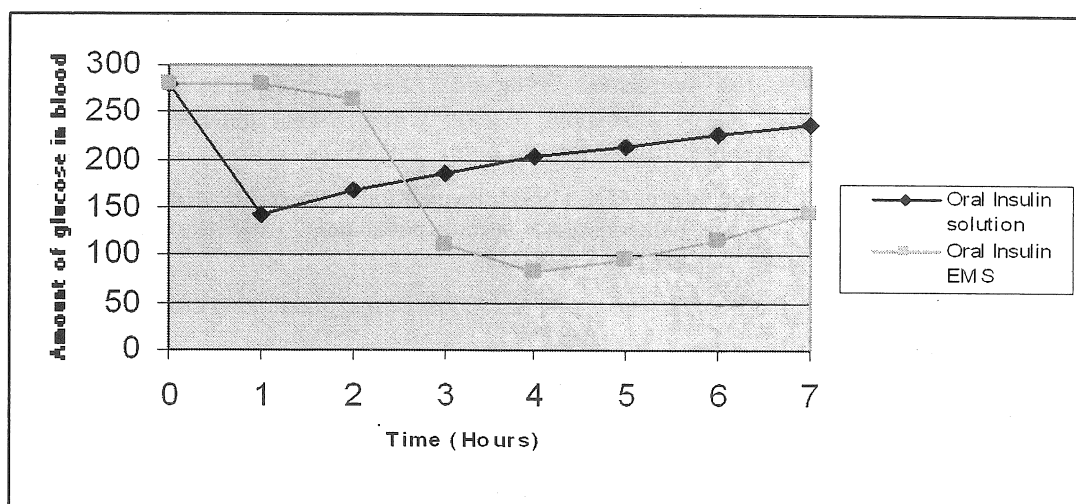| Time in hours	With use of insulin solution	With use of insulin EMS
0	280	280
1	141	279
2	168	265
3	186	112
4	205	84
5	215	96
6	228	117
7	237	145

Figure 3. In-vivo hypoglycemic effects of oral insulin solution and oral insulin EMS.

Conclusions

In this study, progress has been made in the development of oral delivery route for insulin therapy so as to overcome the drawbacks associated with parenteral delivery mechanism. To increase the oral bioavailability, insulin enteric microspheres (Hydroxy propyl methyl cellulose acetate succinate was used as enteric polymer) containing protease inhibitor (Bacitracin) and absorption enhancer (Sodium oleate) were formulated. The results of this study confirm that the formulation can be used successfully for oral drug delivery of insulin because it provides advantages of protection from digestive enzymes in stomach and upper part of GIT, protection from proteolytic degradation and enhanced absorption of insulin in GIT.

References

Bai, J.P. and Chang, L.L. (1996). Effects of enzyme inhibitors and insulin concentration on the transepithelial transport of insulin in rats. *J. Pharm. Pharmacol*. 48: 1078-1082.

Bernkop-Schnurch, A. and Thaler, S. (2000). Polycarbophil-cysteine conjugates as platforms for oral (poly)peptide delivery systems. *J. Pharm. Sci*. 89: 901-909.

Carino, G.P. and Mathowitz, E. (1999). Oral insulin delivery. *Adv. Drug Del. Rev*. 35: 249-257.

DeVries, J.H., Snoek, F.J. and Kostense, P.J. (2002). A randomized trial of continuous subcutaneous insulin infusion and intensive injection therapy I type 1 diabetes for proteins with long-standing poor glycemic control. *Diabetes Care*. 25: 2074-2080.

Donnelly, A., Kellaway, I.W., Taylor, G. and Gibson, M. (1998). Absorption enhancers as tools to determine the route of nasal absorption of peptides. *J. Drug Target*. 5: 121-127.

Herman, W.H., Ilag, L.L. and Johnson, S.L. (2005). A clinical trial of continuous subcutaneous insulin infusion versus multiple daily injections in older adults with type 2 diabetes. *Diabetes Care*. 28: 1568-1573.

Hosny, E.A., Ghilzai, N.M.K. and Elmaza, M.M. (2006). Promotion of oral insulin absorption in diabetic rabbits using pH-dependent coated capsules containing sodium cholate. *Pharm. Acta Helvetiae*. 72: 203-207.

Kotze, A.F., Luessen, H.L., De Boer, A.G., Verhoef, J.C. and Junginger, H.E. (1999). Chitosans for enhanced intestinal permeability: prospects for derivatives soluble in neutral and basic environments. *Eur. J. Pharm. Sci*. 7: 145-151.

Krauland, A.H., Guggi, D. and Bernkop-Schnurch, A. (2004). Oral insulin delivery: the potential of thiolated chitosan-insulin tablets on non-diabetic rats. *J.Control. Rel*. 95: 547-555.

Lin, S.Y., Tnan, Y.L., Lee, C.J. and Weng, C.N. (1991). Preparation of enteric coated microspheres of mycoplasma hyopneuminiae vaccine with cellulose acetate phthalate: 1. Formation condition and micromeritic properties. J. Microencapsulation. 8: 318-325.

Marschutz, M.K., Caliceti, P. and Bernkop-Schnurch, A. (2000). Design and in vivo evaluation of an oral delivery system for insulin. *Pharm. Res*. 17: 1468-1474.

Marschutz, M.K. and Bernkop-Schnurch, A. (2000). Oral peptide drug delivery: polymer-inhibitor conjugates protecting insulin from enzymatic degradation in-vitro. *Biomaterials*. 21: 1499-1507.

Martin, E., Verhoef, J.C. and Mercus, F.W. (1998) Efficacy, safety and mechanism of cyclodextrins as absorption enhancers in nasal delivery of peptide and protein drugs. *J. Drug Target*. 6: 17-36.

Mathiowitz, E., Jacob, J.S., Jong, Y.S., Carino, G.P., Chickering. D.E., Chaturvedi, P., Santos, C.A., Vijayaraghavan, k., Montgomery, S., Bassett, M. and Morrell, C. (1997). Biologically erodible microspheres as potential oral drug delivery systems. *Nature*. 386: 410-414.

Modi, P., Mihic, M. and Lewin, A. (2002). The evolving role of oral insulin in the treatment of diabetes using a novel RapidMist System. *Diabetes Metab. Res. Rev*. 18(Suppl 1): S38-S42.

Morimato, K., Uehara, Y., Iwanaga, K., Kakemi, M., Ohasmi, Y., Tanaka, A. and Nakai, Y. (1998). Influence of absorption enhancer (bile salts) and the preservative (benzalkonium chloride) on mucociliary function and permeation barrier function in rabbit tracheas. *Eur. J. Pharm. Sci*. 6: 225-230.

Morishita, I., Morishita, M., Takayama, K., Machida, Y. and Nagai, T. (1993). Enteral insulin delivery by microspheres in 3 different formulations using Eudragit L100 and S100. *Int.J. Pharm. 91:* 29-37.

Pickup, J., Mattock, M. and Kerry, S. (2002). Glycaemic control with continuous subcutaneous insulin infusion compared with intensive insulin injections in patients with type 1 diabetes: Meta-analysis of randomized control trials. *B.M.J*. 324: 705.

Plummer, D.T. (IIIrd Edn.). The Folin-Lowry method of protein assay. *An introduction to Practical Biochemistry.* Tata McGraw-Hill Publishing Company Ltd. New Delhi, pp. 159-160.

Sakai, M., Imat, T., Ohtake, H. and Otagiri, M.(1998). Cytotoxicity of absorption enhancers in Caco-2 cell monolayers. *J. Pharm. Pharmacol.* 50: 1101-1108.

Schipper, N.G., Varum, K.M., Stenberg, P., Ocklind, G., Lennerlans, H. and Artusson, A.P. (1999). Chitosans as absorption enhancers of poorly absorbable drugs. 3: influence of mucus on absorption enhancement. *Eur. J. Pharm. Sci.* 83: 1465-1469.

Scott-Moncrieff, J.C., Shao, Z. and Mitra, A.K. (1994). Enhancement of intestinal absorption by bile salt-fatty acid mixed micelles in dogs. *J. Pharm. Sci.* 83: 1465-1469.

Shao, Z., Lt, Y., Chermark, T. and Mitra, A.K. (1994). Cyclodextrin as mucosal absorbance promoters of insulin: 2. Effects of β-Cyclodextrin derivatives on α-chymotrypsin degradation and enternal absorption of insulin in rats. *Pharm. Res.* 11: 1174-1179.

Shiling, R.J. and Mitra, A.K. (1991). Degradation of insulin by trysin and alpha-chymotrypsin. *Pharm. Res.* 8: 721-727.

Wu, S.J. and Robinson, J.R. (1991). Transport of human growth hormone across caco-2 cells with novel delivery agent's evidence for P-glycoprotein involvement. *J. Control. Rel.* 62:171-177.

Yamamoto, A., Taniguchi, T., Rikyuu, K., Tsuji, T., Fujita, T., Murakami, M. and Muranishi, S. (1994 a). Effects of various protease inhibitors on the intestinal absorption and degradation of insulin in rats. *Pharm. Res.* 1496-1500.

Yamamoto, A., Umemori, S. and Muranishi, S. (1994 b). Absorption enhancement of intrapulmonary administrated insulin by various absorption enhancers and protease inhibitors in rats. *J.Pharm.Pharmacol.* 46: 14-18.