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RESEARCH PAPER

Potential Applications of Polymeric Microsphere Suspension as Subcutaneous Depot for Insulin

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ABSTRACT

The objective of this investigation was to develop an injectable, depot-forming drug delivery system for insulin based on microparticle technology to maintain constant plasma drug concentrations over prolonged period of time for the effective control blood sugar levels. Formulations were optimized with two well-characterized biodegradable polymers namely, poly(DL-lactide-co-glycolide) and poly-ε-caprolactone and evaluated in vitro for physicochemical characteristics, drug release in phosphate buffered saline (pH 7.4), and evaluated in vivo in streptozotocin-induced hypoglycemic rats. With a large volume of internal aqueous phase during w/o/w double emulsion solvent evaporation process and high molecular weight of the polymers used, we could not achieve high drug capture and precise control over subsequent release within the study period of 60 days. However, this investigation revealed that upon subcutaneous injection, the biodegradable depot-forming polymeric microspheres controlled the drug release and plasma sugar levels more efficiently than plain insulin injection. Preliminary pharmacokinetic evaluation exhibited steady plasma insulin concentration during the study period. These formulations, with their reduced frequency of administration and better control over drug disposition, may provide an economic benefit to the user compared with products currently available for diabetes control.

Key Words: Insulin; PLGA; PCL; Depot; Microsphere; Pharmacodynamics; Pharmacokinetics.

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INTRODUCTION

Since the discovery of insulin (INS) in the 1920's, continuous strides have been made to improve the treatment of insulin dependent diabetes mellitus (IDDM).[1–7] Currently marketed INS formulations have not yet attained the physiological goal of providing constant INS levels to meet the basal needs between meals and during the night. Because of too slow absorption of regular INS and variable absorption of prolonged acting INSs after subcutaneous injection, diabetic patients often experience large excursions in blood glucose and risk hypoglycemic attacks and the long-term complications associated with high glucose levels. Conversely, sustained blood levels of INS have the disadvantage of suppression of glucagon-mediated homeostasis, thus favoring prolonged hypoglycemic episodes and encephalopathy. INS also can degrade locally at the site of injection, thus limiting the duration of action. The average daily requirement of INS for an adult diabetic patient is 0.5–1.0 IU/kg/day. Under fasting conditions, the pancreas secretes about 40 μg (1 U) of INS per hour into the portal vein to achieve a concentration of INS in portal blood of 2 to 4 ng per mL (50 to 100 μU/mL) and in the peripheral circulation of 0.5 ng per mL (12 μU/mL) or about 0.1 nM. After ingestion of a meal, there is a rapid rise in the concentration of INS in portal blood, followed by a parallel but smaller rise in the peripheral circulation. A goal of INS therapy in insulin dependent diabetes mellitus is to mimic this pattern to finally achieve a mean blood glucose of 155 mg/dL and HbA1c (glycated hemoglobin) of 7.2%. As IDDM patients need a relatively constant basal INS supply to attain a near-normal physiological pattern of INS secretion, from the formulation point of view, the goal is to formulate a system which would express a sustained and stable release of INS during a long period after single injection.

Controlled drug delivery using biodegradable polymers is very attractive among current areas of research.[8–15] In the last decade, research in the area of formulation (especially as microparticle-based injectable depot systems) with biodegradable polymers has led to the commercial development of drug delivery systems in Europe, Japan, and the United States. These include Lupron® depot (Leuprolide), Sandostatin LAR® (Octreotide), Suprefact® 3 depot (Buserelin), and Trelstar® depot (Triptorelin). All these products are directed toward hormone replacement therapy, where a single, once-a-month injection using biodegradable polymers for sustained delivery has replaced 30 or more daily injections.[16–20] INS as well as INS-like growth factor has been formulated in a number of different fashions earlier employing biodegradable polymers to achieve controlled release.[21–32] Majority of them have utilized homo- and copolymers of poly lactic acid and poly glycolic acid which have well-establsihed track record as implantable matrices for sustained drug delivery.[33–37] Pulmonary delivery of INS has also been reported using these polymers for control of hyperglycemia.[38] Our objective was to formulate microparticle-based injectable system in similar direction with poly(DL-lactide-co-glycolide) (PLGA) of two different copolymer ratios and molecular weights and compare with those formulated with poly-ε-caprolactone (PCL) another well-characterized biodegradable polymer that is economic, less hygroscopic than the former, and also employed widely to achieve controlled drug delivery.[33,39]

MATERIALS AND METHODS

Materials

INS injection (plain, bovine source; 40 IU/mL) marketed by M/s Knoll Pharma (tradename Bovine INS PL) was purchased. PLGA (copolymer ratios 50:50 and 85:15, both having intrinsic viscosity 0.55 to 0.75 dL/g) were free samples from M/s Birmingham Polymers, USA. PCL (molecular weight 72,000) was procured from Aldrich Chemicals, USA. Streptozotocin was purchased from Sigma Chemical Co., USA. All other chemicals and reagents were of analytical grade and were used as procured.

Animals

Healthy male Wistar rats of 8–10 weeks of age, weighing 200–250 g were procured from Department of Pharmacology, Kasturba Medical College, Manipal. The institutional Animal Ethical Committee of Kasturba Medical College approved the experimental protocol for all the in vivo studies. The animals were maintained under controlled conditions of temperature ($23 \pm 2^{\circ}C$), humidity ($50 \pm 5\%$), and light and darkness (10 and 14 hr respectively) in polypropylene cages filled with sterile paddy husk as bedding material. They were fed balanced diet (Lipton India Ltd.) and water ad libitum.

Formulation of Biodegradable Polymeric Microspheres

INS-loaded PLGA/PCL microspheres were prepared by emulsion-solvent evaporation method.[40] PLGA/PCL of suitable grade and inherent viscosity was dissolved in appropriate volume of methylene chloride (MC) to get a polymer solution of 2.0 to 4.0% w/v. A primary emulsion (w/o) was prepared with aqueous solution (2.5 mL) of the drug as internal phase and polymer solution as the external phase using a high-speed homogenizer. The primary emulsion was allowed to stabilize for about 15 min and then was poured under high-speed stirring into the bulk of the aqueous solution of polyvinyl alcohol (PVA, 0.1 w/v), previously cooled below 10°C. After high-speed stirring for about 3 min to get secondary w/o/w emulsion, it was agitated slowly using a mechanical stirrer for about 4 hr at 25°C. The microspheres formed were collected by centrifugation (3000 rpm for 15 min) followed by filtration using a Millipore® filtration assembly fitted with Sartorius filters (0.45 μm) and washed with distilled water. Finally, they were dried in a vacuum desiccator for suitable time and stored in airtight, amber colored containers under refrigeration.

Determination of Particle Size

The mean diameter and particle size distribution was determined using particle size analyzer (Model CIS 100, Shimadzu, Japan).

Determination of Encapsulation Efficiency

Twenty-five milligrams of drug-loaded microspheres was dissolved in 5 mL of MC. The drug was back-extracted into 10 mL of 0.05 N hydrochloric acid (HCl) by agitation for 30 min. Aqueous layer was separated and was assayed for drug content after suitable time at 269 nm using a UV-visible spectrophotometer. From the data obtained, drug entrapment efficiency was computed.

In Vitro Release Studies

Weighed microspheres containing a known quantity of INS were suspended in phosphate buffered saline (PBS) pH 7.4 in stoppered flasks which were placed in horizontal shaker water bath maintained at 37 ± 1°C at a speed setting of 25 cycles per minute. At predetermined time intervals, microsphere samples were collected from prelabeled flasks and drug content remaining encapsulated within the microspheres was estimated as explained earlier. Amount of INS released was estimated by mass balance and a graph of time vs. cumulative percentage drug released was constructed.

Pharmacodynamic Evaluation

Healthy Wistar rats were made diabetic by intravenous injection of freshly prepared streptozotocin solution in saline (65 mg/kg) and only diabetes-induced rats (blood sugar > 300 mg/dL) were chosen for further study.[41]

Diabetic rats were divided into three groups containing six rats each. First group served as control without any treatment. Second and third groups received 50 IU/kg of microsphere-based formulations. The microspheres were suspended in suitable vehicle (consisting of 0.5% w/v sodium carboxy methyl cellulose, 5.0% w/v mannitol, and 0.1% v/v polysorbate 80 in aqueous solution). The injections were given subcutaneously using a 21-gauge needle. Blood sugar levels of the experimental animals were recorded periodically using a commercially available glucose estimation kit (glucose oxidase method). Percentage reduction in the blood sugar level was used to evaluate the pharmacodynamic potential of the formulations.

Pharmacokinetic Evaluation

Pharmacokinetic studies of INS formulations were carried out in diabetic rats. The treatment modality was similar to the one explained under pharmacodynamic evaluation. Blood samples were withdrawn periodically by sino-arbital vein puncture using heparinized capillaries; plasma was separated by centrifugation and stored in vials under refrigeration until further analysis. Plasma INS levels were estimated by employing reverse-phase HPLC procedure.[42] Briefly, INS was extracted from blood into 0.05 N HCl (with an intermediate extraction into organic phase) and injected into a reversed-phase (ODS, 5 μm, 25 cm × 4.6 mm i.d.) analytical column with the mobile phase being 74:26 volume ratio of 0.2 M sodium sulfate adjusted to pH 2.3 with phosphoric acid and acetonitrile. The eluent was monitored at 214 nm at a flow rate of 1.2 mL/min.

RESULTS AND DISCUSSION

Formulation Development

The method of preparation with optimized process/formulation parameters resulted in formation of discrete, spherical, free-flowing microparticles with appreciable reproducibility with respect to size distribution, entrapment and release of captured drug load. The results of particle size analysis of drug-loaded polymeric microspheres are illustrated in Table 1. The average particle diameter ranged from 37 to 52 μm. This size is desirable for the purpose of forming a drug depot at site of injection upon subcutaneous implantation.

The entrapment efficiency was not appreciably high as the chances of drug loss during secondary emulsification are higher. Increased entrapment efficiencies could be obtained if we take a polymer of lower molecular weight (< 15,000) with free carboxyl end groups (not end capped—synthesized by direct polycondensation method), thus inducing electrostatic interactions between the carboxyl terminals of the polymer and amino terminals of the peptide drug. One also could make attempts to retain INS within internal aqueous phase by providing conditions for immobilization of INS while in-water drying using agents like gelatin.[33]

In Vitro Drug Release Studies

PLGA (50:50), the most hydrophilic among the polymers used to formulate microspheres, released drug load of up to 30%, against PLGA (85:15) releasing 24% and PCL 21% within 1 month. Rates of drug release ranged from 0.1 IU/mL/day to begin with, up to 0.6 IU/mL/day toward the end of study period. Hydrophobic nature of INS and its molecular size would limit its transport through the polymeric matrix during initial phase. Hence, the pathway for drug release of incorporated INS is through pores formed upon increased exposure time to release medium by hydrolytic cleavage of the polymer chain. The release pattern of the drug from the polymeric microparticles can be modulated by manipulating the polymer characteristics (molecular weight and composition). If we formulate similar system with PLGA of 75:25 copolymer ratio and molecular weight 15,000 (as in the case with leuprolide acetate microparticles), we can arrive at a system which would release almost 100% of the drug within 1 month.[43]

Pharmacodynamic Evaluation

All the animals injected with streptozotocin (65 mg/kg i.v.) developed hyperglycemia and the animals showing blood glucose above 300 mg/dL 5 days postinjection were taken for the study. On an average, the INS formulations were injected once the sugar level reached about 540 mg/dL so as to get a magnification in the antidiabetic response. The diabetic rats showed severe loss in body weight upon development of diabetes (up to 30% loss).

The animals belonging to control group showed severe hyperglycemia with progress of time as they were not treated with any formulations. After 15 days of induction of diabetes, the animals were sacrificed as the weight loss was maximal and they became very weak. The group treated with plain INS injection (bovine INS, 40 IU/mL; 50 IU/Kg single s.c. dose) showed a reduction in the blood glucose up to 50% of initial after 2 days. As with the formulations, both the biodegradable microsphere systems had comparable onset and duration of action. For PLGA (85:15) microspheres (see Fig. 1), the onset

Table 1. Physicochemical characteristics of microspheres taken for further in vitro/in vivo investigations.

Polymer	D:P ratio	Mean diameter ± SD (μm)	Yield (%)	Entrapment efficiency (%)
PLGA I	1:2	42.35 ± 15.41	77.52	47.24 ± 2.27
(85:15) II	1:4	46.54 ± 18.32	75.98	50.87 ± 3.55
III	1:8	48.75 ± 16.84	76.58	53.41 ± 2.86
PLGA I	1:2	37.45 ± 11.75	76.45	55.34 ± 4.78
(50:50) II	1:4	40.56 ± 16.78	74.47	57.32 ± 3.46
III	1:8	42.56 ± 18.74	73.68	60.24 ± 2.23
PCL I	1:2	46.17 ± 14.98	78.55	58.76 ± 4.14
II	1:4	49.46 ± 18.42	75.98	56.14 ± 3.54
III	1:8	52.61 ± 18.74	76.58	52.24 ± 2.23

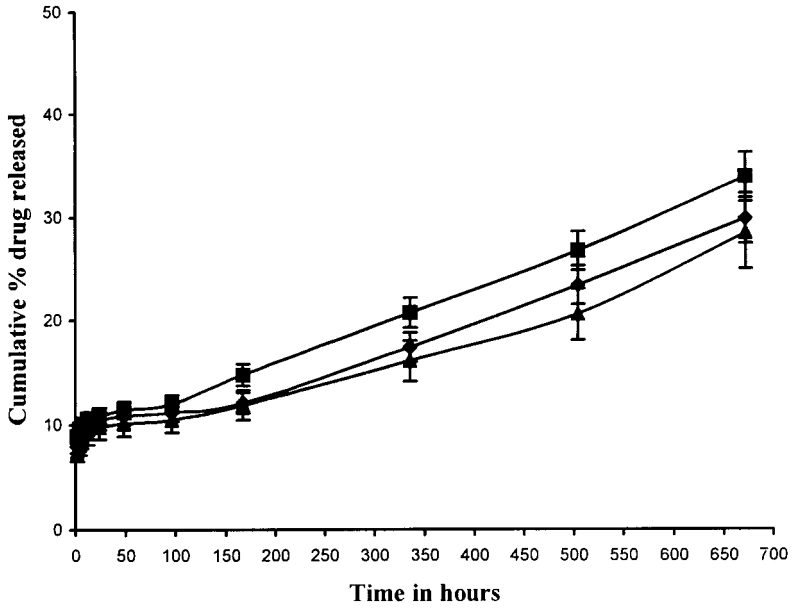

Figure 1. In vitro release profiles of drug loaded microparticles in PBS (pH 7.4). (-■-) INS–PLGA (50:50) formulation. (-◆-) INS–PLGA (85:15) formulation. (-▲-) INS–PCL formulation.

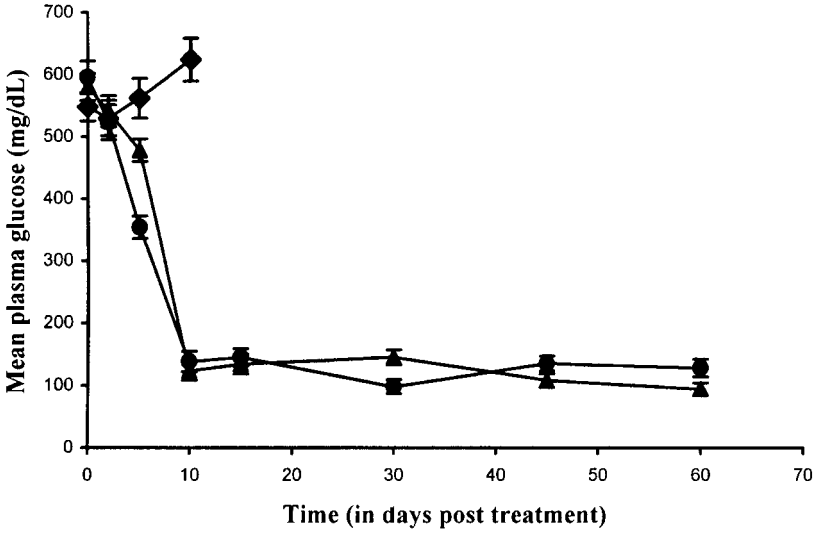

Figure 2. Pharmacodynamic behavior of drug loaded microparticles in diabetic rats. (-◆-) Diabetic control. (-●-) INS–PLGA (85:15) formulation. (-▲-) INS–PCL formulation.

of action (reduction in blood glucose level) was after about 5 days and the maximal action was seen at 30th day sample. Similarly, for PCL microspheres, the onset was after 5 days and maximal reduction in blood sugar was after about 60 days. Both the formulations maintained steady glucose levels in almost normal range (85–110 mg/dL) from 10th day post-treatment up to the period studied (60 days). There was no hypoglycemia seen in the study period. The blood sugar levels remained stable after 10 days postinjection with INS-loaded biodegradable microspheres (refer to Fig. 2).

It can be inferred that the plain injection (the standard formulation used from Knoll) due to its inherent nature of rapid onset of action and shorter duration of action might have reduced blood glucose to much greater extent. However, as the first sample was taken after 2 days post-treatment, the result

obtained does not truly represent the actual extent of reduction in blood glucose levels. It has been reported that INS, even when given as plain injection, gives a tailing (slow release of about 40% of injected dose) up to 3 days.[44] The result obtained for plain INS would represent this fraction of INS, which remains in the circulation due to slow absorption and clearance. The first point of sampling was maintained at the 2nd day for all the formulations so as to get same point of time for comparison and as extrapolated from in vitro studies and polymer characteristics. There was a lag time taken for onset of pharmacological action (about 5 days for both the formulations). The INS was mainly released from the implants thought the pores or the aqueous channels formed in the polymer matrix as it cannot diffuse through the membrane due to its macromolecular nature (MW 5733.6) though other smaller (MW < 1000) water-soluble molecules can. The lag time indicates the time duration taken for the formation of these pores or channels due to the process of hydrolysis of the polymer chains due to imbibed water molecules. PLGA (85:15) has higher percentage of water-insoluble DL-lactide and PCL as such is crystalline and hydrophobic polymer. Hence, both have inherent properties so as to control the release of encapsulated drug. Once the release commenced (i.e., after onset of action), the INS was released though interconnected pores and channels formed due to ongoing bioerosion of the polymeric matrix.

The INS-loaded biodegradable formulations exhibited control of blood sugar for periods ranging for up to 60 days indicating superior pharmacodynamics for INS encapsulated into microspheres. The maximal reduction in glucose level for PLGA microspheres was seen after about 30 days. This could be due to transient surge of INS release from the microspheres (irregularities in channel formation/rate of bioerosion) or variations of time when blood samples were collected under nonfasting conditions might have affected the blood glucose levels. One can expect that higher rates of INS release would be reached with time due to reduction in the molecular weight of the polymer as a result of biodegradation. The incorporated drug payload would be completely delivered as the microparticles lose their structural integrity and polymeric matrix undergoes systematic biodegradation in a controlled manner. We have to switch to the low molecular weight polymers to accelerate the process of termination of drug release as reported recently.[45,46]

Pharmacokinetic Evaluation

Pharmacokinetic evaluation of INS-loaded biodegradable microspheres was done in diabetic rats. Single dose study design was followed up to a period of 60 days post-treatment. It can be seen from Fig. 3 that both the biodegradable formulations did not reach the elimination phase (i.e., where the polymer matrix loses its integrity and begins to bioerode as water-soluble fragments). The plasma INS level was maintained at a steady rate from about 15th day onward for both the formulations. This corresponds to a steady blood glucose level, which is also evident from pharmacodynamic evaluation (Fig. 2). The reasons for maintaining a steady INS level have been explained under the pharmacodynamics section. While correlating the plasma INS levels with blood glucose levels, an inverse relationship could be observed.

The sole pharmacokinetic parameter available for comparison of free INS with the biodegradable formulation was area under the plasma concentration–time curve (AUC). This is because of the extended periods of biodegradation time required for PLGA (85:15) and PCL to biodegrade and to release entrapped INS. Both the biodegradable delivery systems exhibited comparable AUCs (INS-PLGA (85:15) and INS-PCL depots achieved AUCs' of 9756.7 and 9879.3, respectively) during the study period. As we understand, drug concentrations in plasma are no more than a surrogate for pharmacological and clinical effects, and the relevance of this is

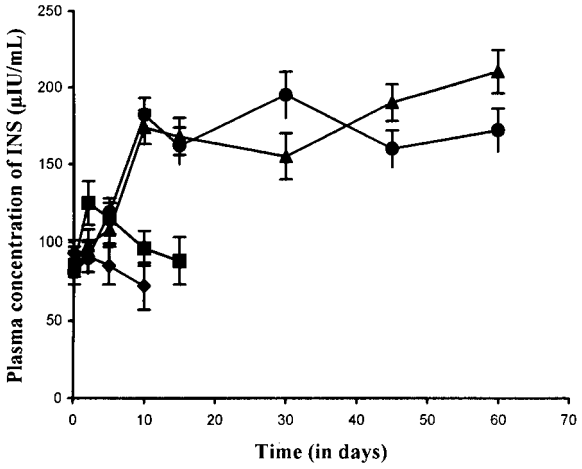

Figure 3. Pharmacokinetic profile of drug loaded microparticles in diabetic rats. (-◆-) Diabetic control. (-■-) INS–plain injection. (-▲-) INS–PLGA (85:15) formulation. (-●-) INS–PCL formulation.

reflected in the pharmacodynamic data presented here. The data generated with this preliminary pharmacokinetic investigation is inconclusive and one needs to study further and estimate the complete disposition profile to translate the quantitative information generated into desired characteristics of the drug release kinetics from the delivery system.

CONCLUSIONS

INS formulation and delivery is presently at the crossroads, and over the next decade dramatic changes in diabetic therapy are likely to take place, many of which will be technology driven. Provided a reliable long duration glucose sensor becomes available, the implantable pump may represent the ultimate solution to optimal INS therapy although the cost will presumably prevent its widespread use. The present investigation represents only a preliminary study to prepare INS for achieving stationary release over a long time after a single depot injection. The long duration is attributed solely to the fact that INS was gradually released with degradation of the polymeric matrix. Hence, one can choose the polymer of right molecular weight and composition to engineer the release profile as per the need. Compared to the ideal flat release profile with time, both in vitro and in vivo release curves showed a lag phase with a gradual escalation. As there have been many recent studies in the similar direction, we see a brilliant future for practical realization of the concept of artificial pancreas achieved via a implantable polymeric system for INS.

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