Magnetite-PLGA Microparticles for Oral Delivery of Insulin

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ABSTRACT

Magnetic responsive particles were designed for use in oral delivery of insulin. Magnetite nanoparticles (12 nm average size) were synthesized and co-encapsulated with insulin into poly(lactide-co-glycolide) (PLGA) microparticles (4.6 ± 2.2 μm average particle size) through the double-emulsion method. The spherical structures of resulting microparticles were well maintained at magnetite content 5 wt % or less. Mice were gavaged with ^{125}I-insulin-magnetite-PLGA microparticles and a circumferential trans-abdominal magnetic field was applied forty minutes after administration to retard the transit of the microparticles in the intestinal tract. As control, mice were similarly dosed without the subsequent trans-abdominal magnetic field. Mice were sacrificed, and the intestinal radioactivity was 101% and 145% higher in treated mice versus the control at 6 h and 12 h, respectively. A single administration of 50 unit/kg Humulin R-magnetite-PLGA microparticles to the fasted mice resulted in 66% reduction of blood glucose level in the presence of external magnetic field at 12 h, compared to 27% reduction in the absence of magnetic field.

INTRODUCTION

Oral delivery of peptides and protein therapeutics has been extensively studied in the past several decades. This route of administration is preferred because it increases patient compliance and comfort compared to the parenteral route, which accounts for the administration of more than ninety percent of FDA approved protein drugs. However, clinically effective oral delivery systems for protein therapeutics have not been established [1].

Proteins administered orally result in extremely poor absorption into the circulatory system due to the degradation of proteins in harsh acidic and enzymatic conditions in the stomach, and low permeation of proteins across the intestinal membranes [1]. Several approaches have been proposed and evaluated for oral delivery of proteins, including permeation enhancers [2-4], pH-sensitive hydrogels [5], enzyme inhibitors [6], liposomes [7-9] and protein-encapsulated sub-micron sized polymeric particles [10, 11].

Polymeric microparticles (MPs) are easy to prepare, encapsulate protein with high efficiency (usually greater than 50%), and effectively protect encapsulated proteins from degradation in gastrointestinal tract (GIT) [1]. The drawback of this approach is that the majority of the particles pass through small intestines without being absorbed. Retention of these protein-containing particles in the small intestine for an extended period of time may result in an increase of the delivery efficiency through either the absorption of localized particles or through the

absorption of protein drugs that are released in small intestine from these particles. Different approaches have been attempted to slow down the intestinal transit of orally administrated drug carriers. For example, mucoadhesive polymeric particles that can adhere to mucus layer in the intestine have been studied [12].

Magnetically modulated particulate systems have attracted much attention recently for use in *in vivo* imaging and in targeted drug delivery. Imaging ligands or drugs can be readily localized at targeted sites through an external magnetic field [13]. We have been developing magnetic particulate carriers that may be localized by an external magnetic field in intestinal areas for effective oral delivery of protein [7]. Herein we report the preparation and study of magnetite containing, insulin-encapsulated polymer microparticles for oral delivery of insulin.

EXPERIMENTAL DETAILS

General

Human insulin (Humulin R, 500 U/mL), a model drug used in this study, was purchased from drugstore.com. Poly(DL-lactide-co-glycolide) (50/50) with acid terminal groups (PLGA, inherent viscosity 0.18 dl/g) was obtained from Absorbable Polymers International (Pelham, AL). Poly(vinyl alcohol) (PVA, MW 30 kDa-70 kDa), Iron (III) chloride and Iron (II) chloride were purchased from Sigma/Aldrich chemical company and used as received. FITC-magnetic polystyrene beads (fluorescent YG superparamagnetic microspheres, 1-2 μm) were purchased from Polysciences, Inc. Scanning electron microscopy (SEM) was recorded on a JEOL JSM 6060 system. Fluorescent images were taken on an Axiovert 200 inverted microscope (Zeiss). Magnets (Neodymium iron boron rare earth magnets, 1" × 1" × 0.5"(thickness), Grade N40, magnetized through the thickness) were purchased from amazingmagnets.com. Radioactivities of ^{125}I-insulin or MPs were analyzed on a TRI-CARB Liquid Scintillation Analyzer (Model 2200CA, Packard Instrument Company, Downers Grove, IL) using 5-10 mL Hionic-Fluor cocktail. Solvable was purchased from Packard Instrument Company. Magnetite nanocrystals (12 nm) were synthesized following the published procedure[14]. BALB/C mice were purchased from Charles River laboratory (Wilmington, MA).

Preparation of PLGA microparticles (MPs) encapsulating Humulin R or ^{125}I-Insulin

Insulin encapsulated MPs were prepared using the water-in-oil-in-water solvent evaporation procedure (double emulsion). 50 μL of the Humulin R solution (500 U/mL) was emulsified with 50 mg PLGA in dichloromethane (1 mL) for 30s using a probe sonicator at 10W. The first emulsion was transferred to a 50 mL aqueous PVA solution (1 % w/v) and homogenized at 8000 rpm for 1 minute. The resulting emulsion was immediately poured into a 150 mL aqueous PVA solution (0.3 % w/v) with gentle stirring. Organic solvent was removed through slow evaporation at room temperature for 2.5 h. The resulting insulin containing MPs were isolated by centrifugation at 4000 rpm and at 10 ^{o}C for 10 minutes, washed twice with double-distilled water and lyophilized. The yields of MPs are in a range of 50-60% with encapsulation efficiency 60-80%. Loading of insulin was determined by protein BCA Assay (PIERCE) by dissolving MPs using a mixture of acetonitrile and water.

The procedures for making ^{125}I-Insulin-encapsulated, magnetite-containing MPs are same as Humulin R-encapsulated MPs except that 50 μL ^{125}I-Insulin solution (5-10 μCi) was used.

Preparation of Humulin R-encapsulated, magnetite-containing PLGA MPs

The procedure for making Humulin R-encapsulated, magnetite-containing MPs is the same as Humulin R-encapsulated MPs except that 2-10 wt% of magnetite (relative to the mass of polymer) was added during the first emulsion. MPs containing 2-5% of magnetite can well maintain their spherical structure (Figure 1B-C). When magnetite content increases to 10% in MPs, the spherical structures of the resulting MPs become less stable (Figure 1D).

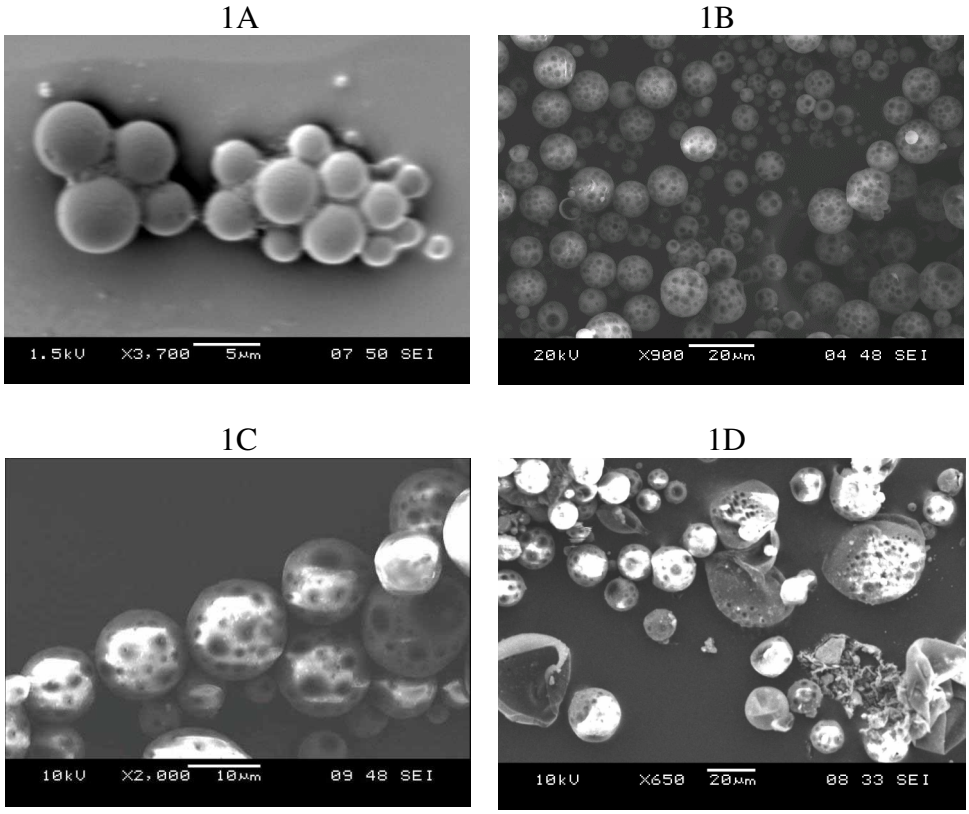

Figure 1. SEM image of Humulin R-encapsulated, magnetite PLGA MP. Magnetite content in weight percentage: A) 0%, B) 2%, C) 5%, D) 10%.

Characterization of insulin containing PLGA MPs

The sizes of MPs were measured on a Beckman Coulter Multisizer^{TM}-3. Electrophoretic mobilities were measured at 25 °C on a ZetaPALS dynamic light scattering system (Brookhaven Instruments Corporation) using BIC PALS zeta potential analysis software. Zeta potentials were calculated using the Smoluchowsky model.

Oral administration of FITC-paramagnetic beads

Mice were fasted for 12 h and then gavaged a solution of fluorescent YG superparamagnetic microspheres (5 mg/ 200 µL PBS). Forty minutes after administration, mice were restrained and a magnet was applied to their abdominal area. Control mice were restrained in the same way but no magnet was applied. Mice were sacrificed 6h after administration. Small intestines of the mice from both groups were collected, dissolved in Solvable and analyzed by fluorescent microscopy.

Oral administration of ^{125}I-Insulin-encapsulated PLGA MPs

Mice were fasted for 12 h and then orally administered with 1 μCi ^{125}I-insulin-magnetite (2 wt%)-PLGA microparticles in 200 μL water. Forty minutes after administration, mice were restrained in the presence or absence of magnetic field. Mice were sacrificed at 6h and 12h. Small intestines were dissolved by Solvable and analyzed using a scintillation counter.

Oral delivery of insulin using Humulin R-PLGA-magnetite (2 wt%)-PLGA MPs

Mice were fasted for 12 h and then orally administered with Humulin R-PLGA-magnetite (2 wt%)-PLGA MPs at 50 unit/kg. Glucose levels were measured using Ascensia Breeze Blood Glucose Monitoring System (Bayer). Four mice were assigned to each group such that the mean values of their initial glucose levels were consistent. Humulin R-PLGA-magnetite (50 unit/kg) in 200 μL PBS were administrated orally using gavage needles. Control mice were administered with 200 μL PBS only. The glucose level of each mouse was monitored over time.

RESULTS AND DISCUSSION

The intestinal transit of particulate drug delivery vehicles is relatively fast in mice. The majority of the particles travel through the intestine in about 2 hours [15]. This rapid transit time prevents delivery vehicles from being absorbed onto the surface of intestinal epithelium. In order to slow down the transit of polymer microparticles, we have developed magnetically responsive polymer particles by incorporating magnetites. The magnetically responsive drug carriers are localized in the intestine by the application of an external magnetic field, resulting in either enhanced absorption of drug carriers or absorption of the locally released protein drugs.

To evaluate whether the external magnet can retain magnetic particles in the intestine, we used fluorescent, paramagnetic beads as a model drug delivery system. The retention of MPs in the intestines is significantly improved in the magnet-applied mice compared to the control mice. The presence of fluorescent particles in the small intestines was visualized using fluorescent microscopy (Figure 2). Mice that are applied an external magnet field showed much stronger fluorescent activity than mice that are not applied an external magnetic field (Figure 2B and 2C).

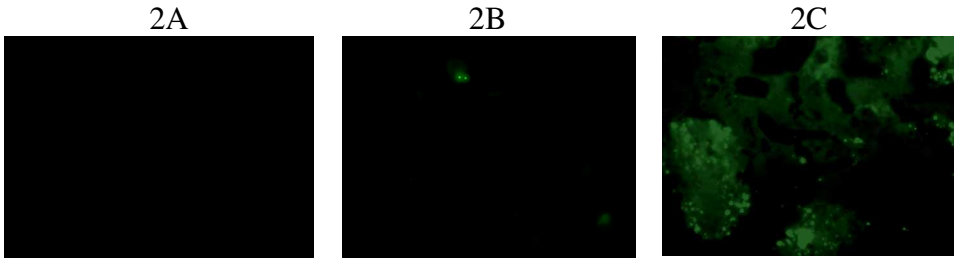

Figure 2. Homogenized small intestine solution of mice in the group of A) no administration of magnetic particle; B) receiving 5 mg of fluorescent YG superparamagnetic microspheres and being restrained for 6h in the absence of external magnet; C) receiving 5 mg of fluorescent YG superparamagnetic microspheres and being restrained for 6h in the presence of external magnet.

Mice gavaged with ^{125}I-insulin-magnetite-PLGA MPs and applied with an external magnetic field have intestinal radioactivitities 101% and 145% higher than the control mice at 6 h and 12 h, respectively (Figure 3A). This striking difference of observed radioactivity clearly shows encapsulated magnetite is responsive to the applied external magnetic field, resulting in an increased retention of MP in small intestines. We further evaluated the efficacy of Humulin R encapsulated, magnetite containing PLGA MPs. A single administration of 50 unit/kg of Humulin R-magnetite-PLGA microparticles to fasted mice resulted in 66% reduction of blood glucose level in the presence of external magnetic field at 12 h, compared to 27% reduction in the absence of magnetic field.

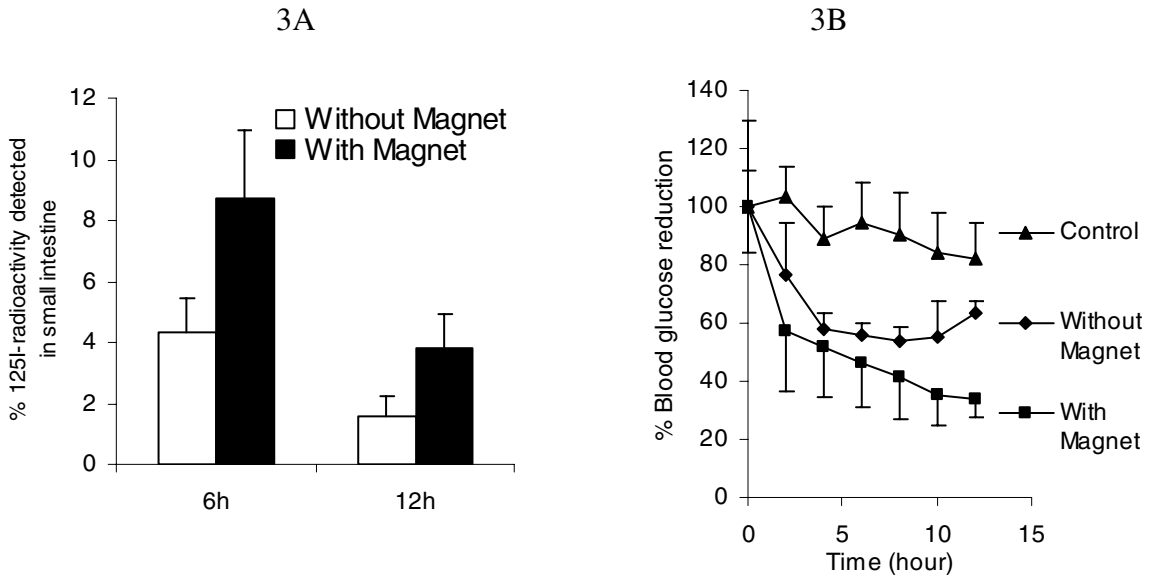

Figure 3. A) Mice treated with 1 μCi ^{125}I-insulin-magnetite (2 wt%)-PLGA microparticles, restrained in the presence or absence of a magnetic field. Small intestines were collected, solubilized and analyzed using scintillation counter at 6 h and 12 h. B) Glucose reduction by Humulin R-magnetite-PLGA microparticles (50 unit/kg) in the presence and absence of magnetic field.

CONCLUSIONS

Magnetic responsive materials were studied for use in oral delivery of insulin. Co-encapsulation of magnetite materials with insulin in PLGA microparticles results in significant retention of the MPs in the intestine with the application of an external magnetic field. The efficacy of this approach was confirmed by showing reduced glucose levels for up to 12 hours post-administration. Our study suggests that design of magnetic responsive delivery vehicles is a promising approach for oral protein delivery.

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