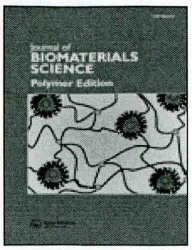

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Extended release peptide delivery systems through the use of PLGA microsphere combinations

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Abstract—The purpose of this study was to evaluate the utility of combining polymer matrices to overcome extended lag periods or unacceptably short durations of action intrinsic in the individual polymer systems. Leuprolide, an LHRH superagonist, was incorporated into a variety of poly(lactide-*co*-glycolide) (PLGA) matrices using a solvent extraction/evaporation method. The *in vitro* release of Leuprolide from these matrices was evaluated at pH 7.0 and 37°C in phosphate buffer. The formulations were administered to an animal model at 3 or 9 mg kg^{-1} doses and serum testosterone levels were followed using a RIA method. A two-part system was made by combining microspheres made from a 75 : 25 acid terminated PLGA and microspheres made from a 75 : 25 ester terminated PLGA. This combination elicited chemical castration from 10–100 days. A three-part combination composed of an ester terminated 75 : 25 PLGA formulation, an ester terminated 50 : 50 PLGA formulation and an acid terminated 50 : 50 PLGA formulation also provided a composite profile with an onset of 10 days and a duration of ~100 days. Additionally, a single polymer system composed of a high molecular weight ester terminated 75 : 25 PLGA was employed to produce release over the desired 90-day release period. This study demonstrates that microsphere combinations can potentially provide effective therapies over extended intervals when combined at the proper ratio.

Key words: Peptide delivery; sustained release; microspheres; poly(lactide-*co*-glycolide); LHRH superagonist; leuprolide.

INTRODUCTION

Delivery of highly potent peptides and proteins pose some interesting challenges for the pharmaceutical scientist. Low bioavailability and *in vivo* stability often preclude conventional formulation. For certain clinical applications, a delivery system that can ensure continuous release of a peptide for longer than 30 days would provide

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a convenient and efficacious system for delivery of these compounds [1–4]. A 90-day duration provides for administration only four times per year providing better patient compliance. Further, from a pharmacoeconomic standpoint, a 90-day dosage form could provide a less expensive alternative to daily or monthly injections.

Aliphatic polyesters, such as poly(lactide-*co*-glycolide) (PLGA) are biocompatable and biodegradable and are therefore good candidates for a controlled delivery system [5–8]. However, because these polymers release higher molecular weight drugs like peptides primarily by an erosional mechanism, there exists the possibility of a lag phase between the diffusional controlled release and the erosional controlled release [9–11]. This presents a challenge for providing continuous release over the entire release period. The lag phase of individual systems could be addressed by combining systems produced from different polymers [12, 13]. In this way, the desired extended continuous release periods could be achieved.

Leuprolide acetate, when released in a continuous manner, significantly reduces serum testosterone levels which has implications in the treatment of several diseases such as prostate cancer, precocious puberty, endometriosis, and mammary cancer [14]. It is a nine amino acid peptide with a molecular weight of ~1200 Da having little or no tertiary structure making it a good candidate for incorporation into these PLGA systems. Leuprolide is a LHRH superagonist exploiting the pituitary–testicular axis to decrease testosterone levels. Under the normal physiology, a burst of LHRH is released from the hypothalamus and travels to the pituitary where it binds a receptor on the surface of a gonadotrope. This stimulates the gonadotrope to release its store of gonadotropin (LH, FSH) as a burst and to begin synthesis of more gonadotropins. LH binds a receptor on the Leydig cells causing testosterone production and release. When LHRH is released in a continuous manner, the receptors on the gonadotrope saturate and the cell is down regulated resulting in release of smaller amounts of less active gonadotropin. This culminates in less stimulation of the Leydig cells and lower testosterone levels. Superagonists, like Leuprolide, have greater serum stability and higher binding affinity for the gonadotrope when compared to native LHRH [15].

The objective of this study was to evaluate the feasibility of combining microsphere systems to produce a 90 day testosterone suppression profile using Leuprolide incorporated into PLGA polymers of varying end groups, monomer ratios, and molecular weights. In order to produce combinations capable of providing the desired 90 day efficacy, many polymer systems were individually evaluated *in vitro* and *in vivo*. Based on the data obtained from the individual systems, several candidates having either prolonged lag phases or short durations were identified for further study in the form of microsphere combinations. These microsphere combinations were subsequently tested for *in vivo* suppression of testosterone.

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MATERIALS AND METHODS

Matrix selection

The matrices selected for evaluation in the form of microsphere combinations were based on the screening of seventeen systems. Each individual system was evaluated for *in vitro* release and *in vivo* efficacy. Systems, which showed shortened duration or extended lag which could be compensated by combining with other systems, were identified based on the individual release and efficacy profiles. Five candidate formulations were selected for combination to produce testosterone suppression through 90 days.

Polymer characterization

Polymers obtained from Boerhinger Ingelhiem (Ridgefield, CT, USA) were used in the preparation of the Leuprolide loaded PLGA microspheres. The PLGAs were characterized for acid number, weight and number average molecular weight, polydispersity, thermal transitions, and cloud points using a number of techniques including gel permeation chromatography, differential scanning calorimetry and titration [16]. The polymer properties are shown in Table 1.

Microsphere preparation

Leuprolide acetate (Bachem, Inc., Torrance, CA, USA) was encapsulated into the various polymers by a solvent extraction/evaporation method [17, 18]. The polymer was dissolved in dichloromethane and the Leuprolide in methanol. The two solutions were then combined to provide the dispersed phase (DP). The DP was added to an aqueous continuous phase (CP) containing 0.1–0.35% poly(vinyl alcohol) under rapid stirring. After the microspheres had partially solidified due to solvent extraction, the temperature of the CP was increased to 40°C for 1 h to remove the remaining solvent and to ensure a low residual solvent content, i.e.

Table 1.
Polymer properties

Polymer	ID	Ratio LA : GA	Molecular weight			T_g[a]	Acid number[b]	Cloud point[c]
			M_w	M_n	PD			
A	RG756	75 : 25	95 285	57 373	1.66	45.3	0.3	32.7
B	RG752	75 : 25	15 577	6541	2.38	42.4	1.1	57.6
C	RG75 : 25H	75 : 25	11 161	5062	2.21	44.5	14.3	69.6
D	RG503H	50 : 50	28 022	13 233	2.12	46.2	4.6	28.0
E	RG502	50 : 50	10 754	5014	2.15	39.8	0.9	35.6

[a] 2nd heating.

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<100 ppm. The temperature of the CP was reduced to 25°C and the microspheres were isolated by filtration using an 8-μm Millipore SC filter. The recovered microspheres were dried in a vacuum oven at RT overnight and subsequently stored in a desiccator at 5°C.

Microsphere characterization

HPLC method for determination of Leuprolide. HPLC was used for the determination of Leuprolide. The chromatography system was a Dionex AS3500 Chromatography system consisting of a quaternary gradient pump, an autosampler, an Advanced Computer Interface, a Variable Wavelength Detector and the Al-450 Chromatography software (all by Dionex, Sunnyvale, CA, USA); and a Bondapak C18 300 × 3.6 mm column with a Bondclone 10 C18 Guard 30 × 3.9 mm guard column from Phenomenex (Torrence, CA, USA). Detection was at 220 nm. Sample concentrations were determined relative to a Leuprolide standard curve. The mobile phase consisted of Milli-Q purified water (Waters, Milford, MA, USA) and HPLC grade Acetonitrile (Fisher Scientific) at a ratio of 68 : 32 v/v with 0.1% Triflouroacetic acid (Fisher) as an ion pairing agent. The mobile phase was degassed by helium purge (10 min) and the reservoir was kept under slight pressure (less than 5 mm Hg) with helium. The flow rate was 1.1 ml min^{-1}. The injection volume was 30 μl for the extraction samples and 100 μl for the release media. The total run time was 8.0 min.

Drug content. Drug content was accomplished by extraction. Ten mg of the microspheres were quantitatively transferred to a 12-ml glass test tube. The matrix was solubilized in 2 ml of dichloromethane, 10 ml of 0.1 M, pH 4.0 acetate buffer added and the tubes were either agitated by a wrist action shaker or rotated on a wheel for 1 h. Samples were centrifuged and the aqueous layer was analyzed by HPLC. A second extraction with 10 ml of acetate buffer was made to ensure complete extraction and effect mass balance.

In vitro *studies*

In vitro release studies were conducted in 10 ml of 0.03 M phosphate buffer, pH 7.0, incubated at 37°C. Individual samples of approximately 10 mg were transferred to screw capped glass tubes for each assay point and the tubes placed on a tube rotator (18 rpm). Because Leuprolide is unstable in the release media, the drug release was based on extractable drug from the microspheres relative to time zero. To accomplish extraction, the tubes were centrifuged at 3000 rpm for 5 min and 8 ml of release media were removed. Extraction of the separated microspheres was accomplished by adding 2 ml of dichloromethane to the release tubes and vortexing to solubilize the matrix. Eight ml of 0.1 M, pH 4.0 acetate buffer were added and the tubes were agitated on a wrist action shaker for 1 h. The samples were then briefly

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