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Controlled delivery of testosterone from smart polymer solution based systems: In vitro evaluation

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Abstract

The objective of this research is to develop injectable polymers solution based controlled release delivery systems for testosterone (TSN), using phase sensitive and thermosensitive polymers. A combination of poly(lactide) (PLA) and solvents mixture of benzyl benzoate (BB) and benzyl alcohol (BA) was used in the phase sensitive polymer delivery system. The effects of solvents system and drug loading on the in vitro TSN release were evaluated. In the case of thermosensitive polymer delivery systems, a series of low-molecular-weight poly(lactide-co-glycolide)–poly(ethylene glycol)–poly(lactide-co-glycolide) (PLGA–PEG–PLGA) triblock copolymers with varying ratio of lactide/glycolide (LA/GA, 2.0–3.5) were studied to control the release of TSN. The effects of varying block length of copolymers 1–4 on the in vitro TSN release were evaluated. Phosphate buffer saline (pH 7.4) containing 0.5% (w/v) Tween-80 was used as in vitro release medium. The amount of the released TSN was determined by an HPLC method. A controlled (zero-order) in vitro release of TSN was observed from both the phase sensitive and thermosensitive polymer delivery systems. Addition of BA (15%, v/v) in solvents system significantly ($p < 0.05$) increased the release rate of TSN (0.33 ± 0.01 mg/ml) from phase sensitive delivery system in comparison to solvent without BA (0.27 ± 0.00 mg/day). Increasing drug loading also increased release rate. In the case of thermosensitive polymer delivery system, increasing the hydrophobic PLGA block length of copolymers significantly ($p < 0.05$) decreased the release rate of TSN. It is evident from this study that the phase sensitive and thermosensitive polymers are suitable for developing prolong-release injectable implant delivery systems for TSN.

Keywords: Testosterone; Hormone replacement therapy; Hypogonadism; Phase sensitive; Thermosensitive; Biodegradable polymers

1. Introduction

Male hypogonadism results from a variety of pathophysiological conditions in which TSN production is diminished below the normal range and the corresponding plasma TSN concentrations are less than 10 nmol/l (300 ng/dl) (Mazer et al., 1992). TSN has been used

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for androgen replacement therapy since 1930s (Wilson and Griffin, 1980). In recent years, there has been much interest in the use of androgen replacement therapy for treatment of a variety of clinical indications (Wang and Swerdliff, 1997). Besides hypogonadism, androgen replacement therapy is also being considered for the treatment of post-menopausal women and individuals in wasting states owing to their infliction with HIV, cancer or chronic infection. The increasing interest in androgen replacement therapy has prompted the development of many androgen preparations including patches, creams, gels, injectables and implants (Zitzmann and Nieschlag, 2000). However, current delivery methods are far from ideal. Intramuscular injections of TSN esters need to be given every alternate week; daily application of TSN patches or gels may cause skin irritation rashes and adhere poorly; poor oral bioavailability and short duration of action after parenteral administration have dictated the need for a depot formulation of testosterone for androgen replacement therapy. Recently, implants and microspheres have been used but an ideal, long-term continuous release delivery system has not been identified (Goldstein et al., 2001). Microspheres have several inherent disadvantages. These include the need for reconstitution before injection, a relatively complicated manufacturing procedure to produce a sterile, stable and reproducible product and the possibility of microsphere migration from the site of injection. The drawbacks of TSN pellet implants in androgen replacement therapy are the requirement for minor surgical skill in using a trocar and cannula for the implantation and the low but unavoidable rate of pellet extrusion as well as the risk of local hematoma formation, inflammation, infection and fibrosis. In contrast, smart polymer-based injectable solution is simple to prepare and forms an implant upon injection.

Smart polymers that display a physiochemical response in nonlinear fashion to external stimuli (temperature, pH, solvent, magnetic field, electric field, ultrasound, etc.) are widely explored as potential drug-delivery systems (Klein, 2000; Kost and Lapidot, 2000). Several approaches have been reported for the delivery of bioactive molecules in controlled and pulsatile manner using polymeric carrier (Kikuchi and Okano, 2002). Biodegradable, biocompatible, phase sensitive and thermosensitive smart polymer-based delivery system offers several advantages because it releases the drug in a predefined controlled manner, can be used for hydrophilic and hydrophobic or small and large molecular weight therapeutic agents, low burst release, low batch-to-batch variation in comparison to implants or microspheres, achieves high drug loading (up to 90%) in comparison to microspheres and ease of preparation.

Injectable and in situ gel forming delivery system utilizes a miscible blend of a water insoluble polymer and a water miscible biocompatible solvent (such as *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), glycofurol, triacetin, ethyl benzoate and benzyl benzoate (Dunn et al., 1990; Shively et al., 1995; Dunn and Tipton, 1997; Eliaz and Kost, 2000; Wang et al., 2003). Phase sensitive smart polymer solution (water-insoluble smart polymer mixed with organic solvents) utilizes the benefit of both hydrophilic and hydrophobic organic solvents. Upon injection into an aqueous tissue environment, the hydrophilic solvent diffuses out of the polymer system, which then precipitates, resulting in a solid implant in vivo (Jackson et al., 2004) from which the drug is released in a controlled fashion.

The use of block copolymers in drug-delivery was first proposed in early 1980s (Pratten et al., 1985). Copolymers consisting of hydrophobic and hydrophilic blocks tend to form micelles in water to reduce free energy mainly from hydrophobic interactions; their aqueous solutions show thermoreversible gelation with temperature changes. Based on their thermosensitivity and biodegradability, the polymeric materials have been utilized for the in situ formation of a drug-delivery depot by injection of the solution into the body (Jeong et al., 1999). The polymers exhibiting properties of reversible thermal gelation are triblock copolymers consisting of A- and B-blocks, arranged as BAB or ABA, where A is PLGA and B is PEG. Aqueous solutions of these polymers undergo a reversible gel–sol transition and form a free-flowing sol at room temperature becoming a transparent gel at body temperature (Jeong et al., 2000; Kim et al., 2001a; Zentner et al., 2001).

In this study, we investigated the phase sensitive and thermosensitive smart polymer-based delivery systems for delivering TSN. The phase sensitive polymer delivery system utilizes the benefits of both hydrophilic and hydrophobic nontoxic organic solvents to achieve desired release profile of TSN. The effects of formulation factors including different solvent systems and varying drug loading on TSN release were investigated. In

the case of thermosensitive polymer delivery system, copolymer compositions were adjusted to control TSN release.

2. Materials and methods

2.1. Materials

Testosterone was purchased from Spectrum Chemical and Laboratory Products Lab (Gardena, CA). Benzyl benzoate and benzyl alcohol were obtained from Sigma–Aldrich (St. Louis, MO). Poly(DL-lactic acid) with an intrinsic viscosity of 0.20 dl/g was purchased from Polysciences Inc. (Warrington, PA). All other reagents used were of HPLC grade. Four kinds of PLGA–PEG–PLGA triblock copolymers were synthesized and characterized in our laboratory and used in this study. Copolymers 1–4 had block lengths (PLGA–PEG–PLGA) of 995–1000–995, 1125–1000–1125, 1350–1000–1350 and 1400–1000–1400) and molecular weights of 2900, 3250, 3700 and 3800, respectively. The numbers 995, 1125, 1350 and 1400 stand for the molecular weight of PLGA blocks and 1000 for PEG block.

2.2. Determination of the saturation solubility of TSN

An excess of TSN was added in 15 ml screw cap glass test tubes containing 10 ml different medium [PBS, PBS containing 0.5% (w/v) Tween-80 and PBS containing 0.5% (w/v) Tween-80 and 5% (v/v) solvent mixture (BB/BA, 85/15%, v/v)]. The tubes were tightly capped and shaken in a shaking incubator-water bath at 37 °C. After 48 h, the test tubes were centrifuged and the supernatants were filtered through 0.45 μm Millipore® filter membranes (Billerica, MA). The amount of TSN in samples was determined by HPLC as described under analysis of samples.

2.3. Preparation and characterization of in situ gel forming phase sensitive and thermosensitive smart polymer drug-delivery system

2.3.1. Phase sensitive polymer delivery system

Polymer solutions were prepared by placing the mixture of polymer (PLA) and solvents (BB and BA) in shaker water bath at 37 °C for 24 h. Different amount of TSN was dissolved in polymer solution by homogenization, using a Silverson homogenizer (East Longmeadow, MA) for 2 min at 8000 rpm. Six formulations were prepared differing in solvent systems and drug loading.

2.3.2. Thermosensitive polymer delivery system

TSN (300 mg/ml) was added to aqueous copolymer solution and homogenized for 2 min at 8000 rpm. The resulting formulations contained 30% (w/v) of TSN. Injectability of polymer formulations was carried out using 1-ml syringe with 21-gauge needle.

2.4. In vitro drug release studies

2.4.1. In vitro release of TSN from phase sensitive polymer delivery system

A 0.5 ml of phase sensitive polymer formulation containing TSN was injected into 10 ml of release buffer (PBS, pH 7.4 containing 0.5%, w/v Tween-80 and 0.025%, w/v NaN_3) in a glass vial. The vial was incubated in a shaker water bath at 37 °C and shaken at 30 rpm for in vitro release study. The release buffer was sampled at intervals and replaced with the same amount of fresh buffer. At 5% (w/v) PLA concentration, the effect of different solvent systems (mixture of BB/BA at different percentage 100/0, 95/5, 90/10, 85/15%, v/v) on the in vitro TSN (200 mg/ml) release were evaluated; using combination of BB and BA (85/15%, v/v), the effect of drug loading (200, 300, 600 mg/ml) on the in vitro TSN release was also evaluated.

2.4.2. In vitro release of TSN from thermosensitive polymer delivery system

The aqueous thermosensitive copolymer formulation containing TSN (1-ml) was placed into a bottle and allowed to gel at 37 °C. The gel was immersed in 100 ml PBS (pH 7.4, containing 0.5%, w/v Tween-80 and 0.025%, w/v NaN_3) for in vitro study. Bottles were continuously shaken at 37 °C and samples were withdrawn periodically. The amount of TSN in the released samples was determined by an HPLC method as described under analysis of samples. The cumulative amount of released TSN was plotted against time to analyze its release behavior and the slope of the plot gave the release rate constant. The effects of varying

block length of copolymers 1–4 on in vitro TSN release were evaluated.

2.5. Analysis of samples

The amount of TSN in the released samples was determined by HPLC following the method of Noggle et al. (1990) with modification. HPLC system was equipped with a binary pump and automatic injector. TSN was separated on a Phenomenex C_{18} column (3.9 mm × 300 mm, 10 μm particle size); methanol–water (70:30, v/v) was used as mobile phase at a flow rate of 1.2 ml/min. The variable wavelength ultraviolet detector was set at 254 nm and the injections volume was 20 μl. The limit of detection was 5 μg/ml and the TSN standard curve was linear over the range of 5–200 μg/ml.

2.6. Data analysis

Statistical comparisons were made using pair *t*-test. The probability value of less than 0.05 was considered as significant.

3. Results and discussion

3.1. Solubility study

TSN has been reported to be very insoluble in water, with an aqueous solubility of only 46.3 μg/ml at 37 °C (Voorspoels et al., 1996). To study the kinetics of TSN release, the aqueous solubility of TSN needed to be increased in order to maintain sink conditions. Some solvents as well as surfactants have been used to increase aqueous solubility and enable in vitro release studies to be conducted, under sink conditions, in a small volume of medium (Kim et al., 2001b; Jay et al., 2002). The solubility of TSN in three different medium are shown in Table 1. In this study, we add 0.5% Tween-80 in PBS (pH 7.4) to increase TSN aqueous solubility. The results showed that the solubility of TSN at 37 °C was increased from 18.62 to 166.01 μg/ml when 0.5% (w/v) Tween-80 was added. The solubility of TSN was further increased to 1202.5 μg/ml in the presence of 5% (v/v) of solvent mixture (BB/BA, 85/15, v/v) and 0.5% (w/v) Tween-80 in PBS (pH 7.4). The sink condition was maintained by addition of Tween-80 and frequent replacement of fresh buffer during the in vitro release experiment.

Table 1
Solubility of TSN in different medium

Medium	Solubility (μg/ml)
PBS	18.62
PBS (0.5%, w/v Tween-80)	166.01
PBS containing 5% (v/v) BB/BA (85/15, v/v) (0.5%, w/v Tween-80)	1202.5

PBS, phosphate buffer saline; BB, benzyl benzoate; BA: benzyl alcohol.

3.2. Injectability test

Syringeability and injectability problems are associated with microspheres, so the ease of injection of a gel into the subcutaneous tissue is an important consideration. Formulations prepared with phase sensitive polymer were tested to see if they were injectable through a 25-gauge needle. The results are shown in Table 2. All formulations containing 5% (w/v) PLA and different proportions of BB/BA were injectable through a 25-gauge needle. Upon increasing percentage of BA present in solvent system, the formulation (drug loading 20%) became easier to inject through the 25-gauge needle. Upon increasing the drug loading from 20 to 60%, the formulation became more difficult to inject; all formulations (drug loading 30%) containing thermosensitive polymers were injectable through a 21-gauge needle.

3.3. In vitro drug release studies

3.3.1. Phase sensitive polymer delivery system

The drug release mechanism from PLA or PLGA-based matrices has been described by several authors (Fitzgerald and Corrigan, 1996; Hsu et al., 1996). Three mechanisms for controlling drug release from these polymer matrices have been confirmed: Fickian diffusion through the polymer matrix, diffusion through water-filled pores (aqueous channels) formed by water penetration into the matrix and liberation by erosion of the polymer matrix (Athanasiou et al., 1996). The actual drug release from these polymer matrices may be controlled by a combination of these three mechanisms. Drug release may be influenced by physicochemical properties of the polymer and the drug, such

Table 2
Formulation compositions, injectability and release rate from phase sensitive smart polymer-based TSN delivery systems

Formulations	DL-PLA (%, w/v)	BB (%, v/v)	BA (%, v/v)	Testosterone (%, w/v)	Injectability (25G needle)	Release rate (mg/day)
1	5	100	–	20	Yes	0.27 ± 0.00[a]
2	5	95	5	20	Yes	0.29 ± 0.01[a]
3	5	90	10	20	Yes	0.31 ± 0.00[a]
4	5	85	15	20	Yes	0.33 ± 0.01[a,b]
5	5	85	15	30	Yes	0.38 ± 0.01[b]
6	5	85	15	60	Yes	0.85 ± 0.02[b]

DL-PLA, DL-Poly(lactide); BB, benzyl benzoate; BA, benzyl alcohol.
[a] Pair *t*-test showed that all the values are significantly different ($p < 0.05$).
[b] Pair *t*-test showed that all the values are significantly different ($p < 0.05$).

as polymer molecular weight (MW), LA/GA copolymer ratio, drug loading percentage, drug solubility as well as matrix fabrication method (Asano et al., 1989; Fitzgerald and Corrigan, 1996).

Fig. 1 shows the in vitro release of TSN from formulations containing different ratios of BB/BA solvent systems (100/0, 95/5, 90/10, 85/15%, v/v). A sustained TSN release without any "burst" effect during 3 months was observed for all the formulations tested. Addition of BA (15%, v/v) in the solvent systems of gel formulations resulted in a greater release of TSN. Release of TSN from the gel formulations with different ratios of BB/BA solvent systems followed zero-order release kinetics ($R^2 \geq 0.98$). Table 2 shows the zero-order drug release rates of TSN. Increasing percentage of BA in solvent systems of gel formulations significantly ($p < 0.05$) increased drug release rates.

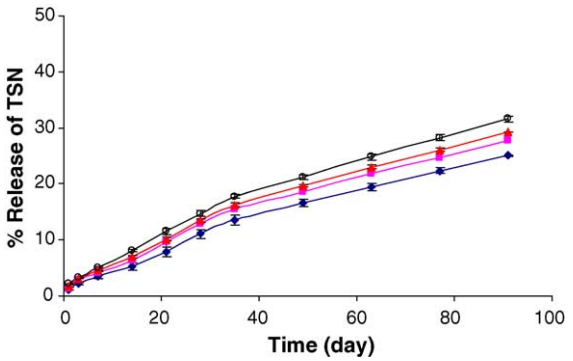

Fig. 1. Effect of varying solvent systems on the in vitro release of TSN from phase sensitive polymer formulations (drug loading 20%). Keys: (♦) benzyl benzoate (100%, v/v); (■) benzyl benzoate (95%, v/v) + benzyl alcohol (5%, v/v); (▲) benzyl benzoate (90%, v/v) + benzyl alcohol (10%, v/v); (◯) benzyl benzoate (85%, v/v) + benzyl alcohol (15%, v/v).

Fundamental parameters of the gel formation kinetics include the water influx rate and the gelation rate. The water influx rate refers to the diffusion of water from the physiologic surroundings and subsequent accumulation within the injected polymer solution. The gelation rate is the rate at which the solution is transformed into a semi-solid, porous implant (Graham et al., 1999). It determines the properties of the diffusional path that the drug molecules take as they leave the implant. In this study, we found greater rate of release from formulations containing greater proportions of BA in solvent mixture, which may be due to faster gelation and/or polymer degradation caused by the presence of greater amount of hydrophilic fraction in the solvent mixture.

Many solvents such as NMP, DMSO, glycofurol, triacetin have been investigated for this delivery system; however, the effect of solvent choice was not clearly defined. Most organic solvents are poorly tolerated and cause pain and local myotoxic effect at the injection site (Hatefi and Amsden, 2002). BB and BA are nontoxic and widely used in injectable formulations. The approach taken here relies on the optimization of solvent properties used for preparation of the in situ forming gels. More hydrophobic solvents such as BB is thought to be less irritating; more hydrophilic solvent BA has local anesthetic effects (Packhaeuser et al., 2004). Using combination of BB/BA may hold some promise to reduce local toxicity in the skin.

Fig. 2 shows the release of TSN from gel formulations with different drug loading. As the drug loading increased from 20 to 60%, the cumulative released amount of TSN also increased. Table 2 shows that zero-order drug release rates were obtained for 3 months

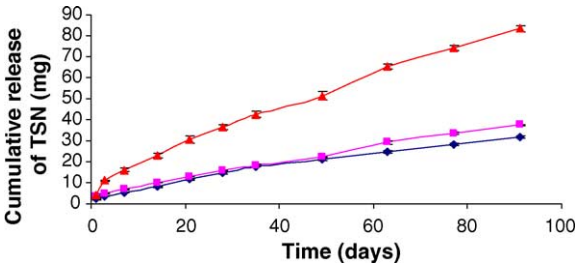

Fig. 2. Effect of varying drug loading on the in vitro release of TSN from phase sensitive polymer formulations. Keys: (♦) 200 mg/ml; (■) 300 mg/ml; (▲) 600 mg/ml.

from all the formulations. Increasing drug loading in the formulations significantly ($p < 0.05$) increased drug release rates. Higher drug density would cause a low polymer density in the matrix, thereby would be reducing the diffusional barrier. Increased loading provides simpler pathways, lower tortuosity and greater porosity for diffusion and facilitates the movement of water into and drug out of the matrix.

3.3.2. *Thermosensitive polymer delivery system*

The in vitro release of TSN from PLGA–PEG–PLGA triblock copolymer matrices with different block lengths was investigated. Fig. 3 shows the in vitro release of TSN from formulations containing different block lengths of copolymers 1–4. Increasing the PLGA block lengths of copolymers resulted in lesser TSN release. A linear relationship of the cumulative release with time shows zero-order release kinetics ($R^2 \geq 0.99$). Table 3 shows that all formulations maintained a release rate of TSN in the range of 1.67–2.18 mg/day for 3 months. Increasing the block lengths of copolymers significantly ($p < 0.05$) decreased the release rate of TSN through the gel formulations.

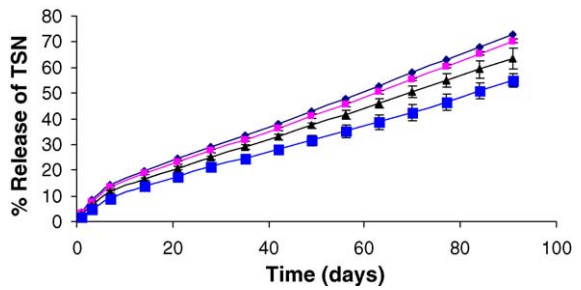

Fig. 3. Effect of varying block lengths of copolymers on the in vitro release of TSN from thermosensitive polymer formulations. Keys: (♦) 30% (w/v) copolymer 1; (small pink square) 30% (w/v) copolymer 2; (▲) 30% (w/v) copolymer 3; (big blue square) 30% (w/v) copolymer 4 (for interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Table 3
Release rate for TSN from thermosensitive polymer delivery systems with varying block lengths of copolymers

Formulation (PLGA–PEG–PLGA)	Release rate (mg/day)
995–1000–995	2.18 ± 0.03[a]
1125–1000–1125	2.11 ± 0.01[a]
1350–1000–1350	1.93 ± 0.12[a]
1400–1000–1400	1.67 ± 0.11[a]

Results have been expressed as mean ± S.D. ($n = 4$).
[a] Pair *t*-test showed that all the values are significantly different ($p < 0.05$).

The advantage of block copolymers as delivery systems is that they may be tailor-made (e.g., size, morphlogy, hydrophobicity and polarity of core) to suit a particular application by changing the properties of the copolymer (e.g., block composition, block length and block ratio). Drug is speculated to release from polymer matrices by a combined mechanism of drug diffusion and polymer erosion (degradation). We found slower release rates from formulations containing longer PLGA block lengths of copolymers, which may be due to slower polymer degradation caused by the presence of greater amount of hydrophobic fraction of LA. Similar findings were reported from other studies on different drugs and systems (Sung et al., 1998; Matsumoto et al., 1999). Sung et al. (1998) found that a greater drug release rate was observed for matrices with lower LA/GA ratio copolymer from PLGA implant. Matsumoto et al. (1999) synthesized various PLA–PEG–PLA triblock copolymers to study the effect of the polymer composition on the progesterone release and found that drug release from the nanoparticles could potentially be controlled by changing the total MW of the copolymers.

4. Conclusions

This study provides evidence that the drug release rates can be controlled by optimizing the formulation factors such as drug loading and solvent composition

in the phase sensitive and block length of triblock copolymers in the thermosensitive delivery systems. Thus, phase sensitive and thermosensitive polymers are good candidates for development of long-acting controlled release injectable solution delivery systems for TSN.

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