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Novel self-assembled tacrolimus nanoparticles cross-linking thermosensitive hydrogels for local rheumatoid arthritis therapy

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ABSTRACT

The aim was to explore the potential application of novel self-assembled nanoparticles cross-linking thermosensitive hydrogels composed of polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol (Soluplus) and tacrolimus (FK-506) for local therapy of rheumatoid arthritis (RA). The sol-gel transition temperature ($T_{sol-gel}$), gelation time, rheological behaviors, in vitro release, in vivo gelation and retention, and therapeutic efficacy against adjuvant-induced arthritis (AIA) rats were compared between the Soluplus hydrogels and widely studied poloxamer 407 (P407) delivery systems. In sol, the spherical and uniform FK506 loaded Soluplus nanoparticles (Soluplus-SNPs) were self-assembled with encapsulation efficiency of 99.5 ± 1.5% and particle size of 73.9 ± 2.9 nm. The decreased $T_{sol-gel}$ of Soluplus-SNPs hydrogels was associated with the addition of salts, elevation of pH and ionic strength. The optimal $T_{sol-gel}$ of Soluplus-SNPs with concentrations of 10%–30% in phosphate buffer (50 mM, pH 7.4) was from 37.4 ± 0.1 °C to 32.8 ± 0.3 °C and the gelation time was not greater than 2 min. Soluplus-SNPs gelling systems showed lower viscosity and wider range concentrations in sol state at 25 °C and stronger gel strength at 37 °C than P407, which resulting in longer sustained release of FK506 but without burst-release in vitro, and longer retention time in the local injection site in vivo. The therapeutic efficacy to treat AIA rats was significantly enhanced from d10 to d17 after a single dose of FK506 loaded in 10% and 20% Soluplus-SNPs hydrogels. In conclusion, Soluplus-SNPs hydrogel is a potential sustainable delivery system for FK506 to treat RA locally.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes chronic inflammation of the synovium, resulting in synovial hyperplasia, bone destruction in the joints and disability. In addition, around 80% of RA patients have one or more comorbidities, especially the acute cardiovascular events, leading to higher mortality and shorten life span [1,2]. Early diagnosis and aggressive treatment to minimize the morbidity associated with its progression are important for RA therapy.

Tacrolimus (FK506) is an immunomodulatory and anti-inflammatory agent by diminishing the ability of calcineurin to dephosphorylate and translocating the nuclear factor of activated T cells that initiate gene transcription for the synthesis of inflammatory cytokines such as tumor necrosis factor, interleukin-2, and interferon-γ. It shows 100-fold more potent in inhibiting T cells proliferation than cyclosporine and becomes a promising treatment for RA in clinic [3–5]. However, poor solubility in water and serious side effects (nephrotoxicity and global immunosuppression) by systemically administration limit its use in clinical practice.

Sustained release formulations with local delivery characteristics can help achieve highly local drug concentrations, while minimize systemic toxicity and decrease the dosing frequency. Both Intra-articular and subcutaneous administrations are common in RA clinical treatment. Compared with intra-articular, subcutaneous injection (SC) is a safer self-administration route for sustained release microspheres and protein drugs [6,7]. It has been reported that subcutaneous tocilizumab demonstrated the sustainable efficacy in most patients and kept the same activity as intravenous injection [8,9]. Also subcutaneous methotrexate was more effective and showed better tolerability than oral administration, potentially avoiding or delaying the

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requirement for future biological treatment [10–12]. In addition, PNIPAAm/EAB-PPPs polymeric micelles loading indomethacin [13] and nano-complexes composed of 1% hyaluronic acid and polyethylene glycol (PEG)-derivatized TRAIL [14] showed sustainable and improved therapeutic efficacy for RA.

Injectable thermosensitive hydrogels, such as poloxamer 407 [15], chitosan [16] and heparin-poloxamer [17], are promising local delivery systems, with sol–gel phase transition in response to changes of temperature. They are syringeable sols at room temperature with minimal invasion during administration, and then become semisolid gels at the local injection sites. To avoid the burst drug release and extend the release duration time, microspheres or nano-complexes in Pluronic® F127 [18,19], microspheres-loaded hydrogel [20], and mPEG-PCL(2000-1800) polymeric micelles in mPEG-PCL-mPEG(550-2200-550) thermosensitive hydrogels [21] have been developed.

Soluplus®, a novel graft copolymer consisting of a polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol in the form of amorphous solid dispersions [22–24] or micelles [25,26] has been widely applied to improve the solubility or dissolution rate of some drugs. Moreover, Soluplus showed excellent self-assembly properties to form nanoparticles(Soluplus-SNPs) at the room temperature and self-crossing to form hydrogels at higher temperature. Therefore, the objective of this study was to develop novel self-assembled nanoparticles cross-linking thermosensitive hydrogels and load FK506 as a locally sustainable delivery system for RA therapy. In this study, the formulations, properties of in vitro and in vivo, and therapeutic efficacy against RA of the novel Soluplus-SNPs hydrogel systems were thoroughly examined and compared with the widely reported poloxamer 407 (P407) delivery system.

2. Materials and methods

2.1. Materials

Tacrolimus was purchased from Huadong medicine Co, Ltd (Hangzhou, China). Soluplus® and Kolliphor®P407 were kindly provided by BASF (Ludwigshafen, Germany). Methanol (HPLC grade, Tedia Company Inc, Ohio, USA). All other reagents were of analytical grade and used without further purification.

ICR mice (male, 6–8 weeks) and Sprague dawley rats (male, 6–8 weeks) were purchased from Zhejiang Academy of Medical Sciences. All animals were fed and maintained under constant conditions at temperature of 20–25 °C, humidity 55 ± 5%, with 12 h light and 12 h dark cycles. Water and food were accessible to animals ad libitum. All studies were performed with the approval of the Institutional Animal Ethics Committee of Zhejiang University of Technology (Hangzhou, China).

2.2. Preparation of various thermosensitive hydrogels

The blank thermosensitive hydrogels containing Soluplus or P407 were prepared by cold method, as described previously [27,28]. In brief, the calculated amount of Soluplus or P407 and water for injection containing salts or pH adjusters were placed in a flat bottom vial and maintained at 4 °C until the homogeneous sols formed. The effects of polymer concentrations, pH values, types of salts, and ionic strength on the properties of the self-assembled nanoparticles, gelatin temperature and time were examined respectively.

FK506 or rhodamine 6G was dissolved in 5 mL ethanol, and then injected into 95 mL water phase containing Soluplus or P407 with continuous stirring at 300 rpm for 30 min to obtain the sols with 3 mg/mL of FK506 or rhodamine 6G.

2.3. Characteristics of self-assembled nanoparticles and thermosensitive hydrogels

2.3.1. Encapsulation efficiency, particle size and morphology of self-assembled nanoparticles

Free FK506 and nanoparticles were separated by microfiltration membranes with the pore size of 0.45 μm. Nanoparticles were dissolved by methanol and FK506 was assayed by HPLC, as described below. Encapsulation efficiency was calculated according to equation: $EE = \frac{W_{FK506-loaded-nanoparticles}}{W_{total-FK506}} \times 100\%$.

The particle size and zeta potential of nanoparticles were determined by dynamic light scattering with a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK) using a 633 nm He-Ne laser beam with a fixed scattering angle of 90°.

The morphology of nanoparticles was observed by transmission electron microscopy (TEM, JEM-1200EX, JEOL, Tokyo, Japan) using negative staining with phosphotungstic acid (PTA, 1.0% w/v).

2.3.2. Gelation temperature and time

A vial inversion method [29] was employed to determine the occurrence of sol-to-gel transition in a water bath from 15 °C to 50 °C at a heating rate of 1 °C per min. The gelation temperature ($T_{sol-gel}$) was recorded after the vial was inverted at an angle of 60° and Soluplus-SNPs or P407 solutions stopped for 30 s. The gelation time at 37 °C was also measured.

2.3.3. The rheological properties

The viscosity of 4 mL sols containing Soluplus 10%, 15% and 20%(w/v) or P407 20%(w/v) in phosphate buffer (50 mM, pH 7.4) was tested by the viscometer (Brookfield DV-II + Pro, Massachusetts, USA) with the probe of SC4-16 (60–1.2 M mPa s) at various temperatures and speed rates.

2.3.4. In vitro release

FK506 in vitro released from the hydrogels containing Soluplus-SNPs 10%, 15% and 20%(w/v) or P407 20%(w/v) were measured by membraneless method as described previously [30,31]. The concentration of FK506 in gels was 3 mg/mL. Samples (0.2 mL) were placed in each test tube (φ = 10 mm) and allowed to gelation at 37 °C for 5 min. The releasing medium was 4 mL phosphate buffer (50 mM, pH 7.4) containing 0.5%(w/v) polysorbate 80, preheated to 37 °C, and carefully added. The in vitro release was performed in a shaking water bath at 100 rpm and 37 °C. The medium (1 mL) was withdrawn at the preset times and replaced with 1 mL of fresh medium. FK506 was assayed by HPLC method [32] with the mobile phase composed of water and methanol (20:80, *v*/*v*) at a flow rate of 1 mL/min and the absorption wavelength of 220 nm. Each formulation was tested in triplicate.

2.4. In vivo gelation and retention

Soluplus-SNPs (10% and 20%, 0.2 mL) or P407 (20%, 0.2 mL) containing 3 mg/mL FK506 or rhodamine 6G were subcutaneously injected into the back of mice (ICR, male, body weight 30 ± 5 g, Zhejiang Academy of Medical Sciences). The skin at the injection site was surgically opened at 5 min, 6 h, 18 h, 24 h, 3 d and 7 d post-administration. The residual gels were pictured, collected and dissolved in 1 mL methanol, and FK506 was assayed by HPLC method described in 2.3.4.

2.5. Therapeutic efficacy against AIA

2.5.1. Adjuvant-induced arthritis (AIA) in rats

Arthritis was induced by inoculation of the Freund's complete adjuvants (CFA). Briefly, on day 0, rats were intradermal injected