European Journal of Pharmaceutics and Biopharmaceutics 130 (2018) 39–47

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Dual-functional lipid polymeric hybrid pH-responsive nanoparticles decorated with cell penetrating peptide and folate for therapy against rheumatoid arthritis

Jinlong Zhao[a], Xueyan Zhang[a], Xiangshi Sun[a], Menghui Zhao[a], Changhui Yu[a], Robert J. Lee[a,b], Fengying Sun[a], Yulin Zhou[a], Youxin Li[a,*], Lesheng Teng[a,*]

[a] School of Life Sciences, Jilin University, Changchun, Jilin 130012, China
[b] College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

ARTICLE INFO

Keywords:
Folate receptor
Polyketal
Cell-penetrating peptide
Lipid polymeric hybrid nanoparticles

ABSTRACT

Methotrexate (MTX), as a disease modifying antirheumatic drug (DMARD), was first line drug to treat rheumatoid arthritis. However, the severe side effect during long term and high dosage usage limit its application. The aim of this study was to develop dual-functional lipid polymeric hybrid pH-responsive nanoparticles to deliver MTX to inflamed joints selectively. The designed MTX loaded stearic acid-octa-arginine and folic acid decorated poly lactic-co-glycolic acid (PLGA) -PK3-based lipid polymeric hybrid nanoparticles (Sta-R8-FA-PPLPNs/MTX) were composed of PK3, Folate-PEG-PLGA, egg PC, and Sta-R8. The nanoparticles exhibited smooth spherical morphology and particle size of 100–150 nm. The in vitro release study indicated that MTX was released faster in phosphate buffered solution (PBS) of pH 5.0 than that in PBS of pH 7.4 from Sta-R8-FA-PPLPNs/MTX. The cellular uptake study revealed that Sta-R8-FA-PPLPNs/MTX were internalized through folate receptor mediated endocytosis into activated macrophages. Therapeutic effects on adjuvant-induced arthritis (AIA) rats further confirm that Sta-R8-FA-PPLPNs/MTX could be promising against rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease associated with synovial hyperplasia and bone erosion [1,2]. RA affects approximately 1% of the adult population and leads to significant morbidity and mortality [3,4]. Besides, RA also increase financial burden to families and society [5]. Though there is no clear-cut cause of RA, activated macrophages have been shown to contribute to inflammation and progressive joint destruction by secreting inflammatory factors [6]. Due to the abundance of folate receptor β (FRβ) on the surface of activated macrophage, drug delivery systems targeting FRβ have been exploited for targeting these cells [6,7]. Since 1980s, methotrexate (MTX), as a disease modifying antirheumatic drug (DMARD), has been the first-line medicine for the treatment of RA [8]. However, RA patients require continuous and long term use of MTX, which induces drug resistance. Though therapeutic effect could be achieved through elevating the dosage of MTX, this strategy is associated with severe side effects [9]. Therefore, delivery MTX to infected joints efficiently became a great challenge

Lipid polymeric hybrid nanoparticles (LPNs), which combine the advantages of liposomes and polymeric nanoparticles, have been extensively studied in recent decades [10,11]. However, LPNs have limited ability to penetrate cell membranes due to its high molecular weight [12,13]. Introduction of cell-penetrating-peptides (CPPs) to LPNs can improve their ability of cell penetration [14,15]. In previous studies, hydrophobic derivatives of octa-arginine with stearic acids (Sta-R8) was applied to deliver siRNA due to its strong siRNA binding, protection, delivery efficiency and low cytotoxicity [16]. There are a number of pH-responsive drug delivery systems have been exploited in many diseases, such as cancer, inflammatory diseases, and

Abbreviations: RA, rheumatoid arthritis; MTX, methotrexate; DMARD, disease modifying antirheumatic drug; LPNs, lipid polymeric hybrid nanoparticles; CPPs, cell-penetrating-peptides; Sta-R8, hydrophobic derivatives of octa-arginine with stearic acids; PK, polyketal; PDI, polydispersity index; DLS, dynamic light scattering; SEM, scanning electron microscope; HPLC, high-performance liquid chromatography; PBS, phosphate buffer solution; FBS, fetal bovine serum; DAPI, 4′,6-diamidino-2-phenylindole; LPS, lipopolysaccharide; CLSM, confocal laser scanning microscopy; AIA, adjuvant-induced arthritis; FA-PPLPNs/MTX, MTX loaded folic acid decorated poly(lactic-co-glycolic acid)-PK3-based lipid polymeric hybrid nanoparticles; Sta-R8-PPLPNs/MTX, MTX loaded stearic acid-octa-arginine decorated poly(lactic-co-glycolic acid)-PK3-based lipid polymeric hybrid nanoparticles; Sta-R8-FA-PPLPNs, MTX loaded stearic acid-octa-arginine and folic acid decorated poly(lactic-co-glycolic acid)-PK3-based lipid polymeric hybrid nanoparticles

* Corresponding authors.
E-mail addresses: liyouxin@jlu.edu.cn (Y. Li), tenglesheng@jlu.edu.cn (L. Teng).

https://doi.org/10.1016/j.ejpb.2018.06.020
Received 5 January 2018; Received in revised form 13 June 2018; Accepted 17 June 2018
Available online 19 June 2018

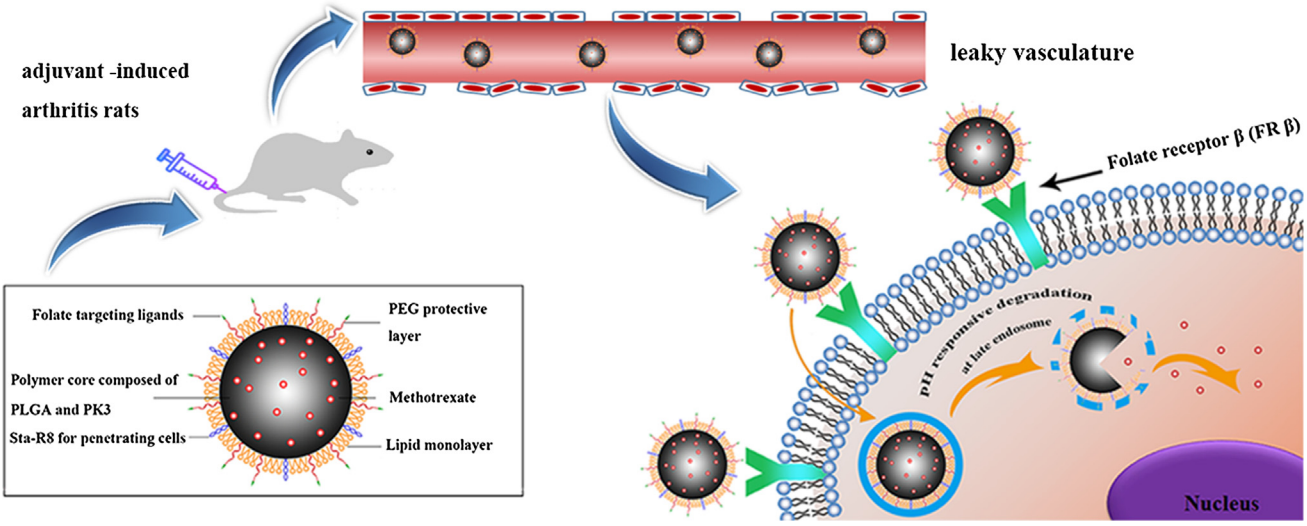

Fig. 1. Schematic illustration of Sta-R8-FA-PPLPNs/MTX in treatment of AIA rats.

cardiovascular system disease [17–20]. PK3, as a novel polyketal (PK), has been widely utilized because of its short hydrolysis half-life at pH 4.5 [21].

To efficiently deliver MTX to inflamed joints, we incorporated PK3, stearic acid- octa-arginine (Sta-R8), Folate-PEG-PLGA, and lipid to synthesize multifunctional nanoparticles. We hypothesized that folate allows for specific binding to FRβ on the surface of activated macrophages, Sta-R8 allows for penetrating macrophages, and hydrophobic core of PLGA/PK3 serves as the pH sensitive switch (Fig. 1).

2. Methods

2.1. Preparation of nanoparticles

PK3 was synthesized as described previously [20,21]. Sta-R8 decorated folate receptor targeting LPNs were synthesized from PK3, Folate-PEG-PLGA, lipid, and Sta-R8 using the following method. Firstly, PK3, Folate-PEG-PLGA, Egg PC, Sta-R8 and MTX were added to a mixed solvent of dichloromethane and acetone followed by sonication to facilitate dissolution. Then, the solution was slowly injected into 1% (w/v) PVA in an ice bath within 30 s and emulsified for 3 min using a probe type sonicator (JY 92-IIN, SCIENTZ BIOTECHNOLOGY, Ningbo, China) at 200 W. The resulting emulsion was then poured into 15 mL of ultrapure water and stirred for 4 h to extract organic solvent. The obtained suspension were centrifuged at 18,000 rpm for 15 min at 4 °C to collect nanoparticles. The precipitate was washed with ultrapure water to remove residual PVA. The obtained nanoparticles were lyophilized in a Virtis ADVANTAGE AD 2.0 freeze dryer (SP Scientific, USA). The resulting nanoparticles powder was kept at 4 °C for further use.

2.2. Characterization of nanoparticles

Average diameter, polydispersity index (PDI) and zeta potential were determined by dynamic light scattering (DLS) on a Zetasizer Nano ZS90 from Malvern (Worcestershire, UK). The nanoparticles were diluted with distilled water to 1 mg/mL and sonicated for 30 s before measurement.

The morphology of various LPNs was investigated by scanning electron microscope (SEM) on a JXA-840 from JEOL (Tokyo, Japan) operating at a voltage of 3 kV. Briefly, the nanoparticles were suspended in ultrapure water at a concentration of 1 mg/mL. Then the nanoparticle suspension was dropped on a silicon wafer. The wafer was left to dry overnight prior to measurement.

The drug loading of MTX in various LPNs was detected using high-performance liquid chromatography (HPLC) method. Firstly, 2 mg of the MTX loaded LPNs were dissolved in 200 μL of dichloromethane. Then 1 mL of phosphate buffer solution (PBS, 0.1 M, pH 7.4) was added to extract MTX followed by centrifuged at 8000 rpm for 10 min at room temperature. The supernatant was collected and filtered (0.45 μm). The obtained solution was injected and detected by HPLC with Breeze system using Extend C18 column (4.6 mm × 250 mm, 5 μm, Agilent Technologies, Inc., Santa Clara, CA, USA). The mobile phase consisted of 2% Na_2HPO4, 7% citric acid, and acetonitrile at a ratio of 80:10:10 (v/v/v). The system was run at a flow rate of 1 mL/min. The following equation was used for the calculation of drug loading.

$$DL \quad (\%) = \frac{amount \quad of \quad drug \quad encapsulated \quad in \quad LPNs}{amount \quad of \quad nanoparticles} \times 100\% \tag{1}$$

2.3. Stability study

Stability of nanoparticles in vitro and in vivo was crucial for application. To determine the stability of nanoparticles under different conditions. Nanoparticles were suspended in the following medium: ultrapure water, PBS of pH 7.4 and PBS of pH 7.4 with 10% fetal bovine serum (FBS). All the above nanoparticle suspensions were incubated at 4 °C and 37 °C to simulate storage condition and in vivo condition. The size and PDI of LPNs were determined using DLS.

2.4. Hemolysis assay

Since the nanoparticles were designed for intravenous injection, a hemolysis assay was carried out according to described previously [22]. Briefly, fresh whole rat blood was obtained from the orbit. The blood sample was then centrifuged at 4000 rpm for 15 min at 4 °C and washed with commercial saline (0.9% NaCl) for three times. Finally, the red blood cells (RBCs) were diluted to 2% (v/v) with saline. LPNs with a series of concentration of MTX were added to the prepared RBCs solution. The mixtures were then incubated in water bath at 37 °C for three hours. Saline and 1% Triton X-100 was used as negative control and positive control, respectively. The suspensions of RBCs were then centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was determined at 540 nm. For calculation of hemolysis rate, we used the following equation:

$$\text{Hemolysis} \quad \text{rate} \quad (\%) = \frac{A_{sample} - A_{negative \quad control}}{A_{positive \quad control} - A_{negative \quad control}} \tag{2}$$