Journal of Controlled Release 283 (2018) 113–125

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Vitamin A-decorated biocompatible micelles for chemogene therapy of liver fibrosis

Jian-Bin Qiao[a,b,c,d,1], Qian-Qian Fan[a,1], Lei Xing[a,b,c,d,1], Peng-Fei Cui[a,b,c,d], Yu-Jing He[a], Jing-Cheng Zhu[a], Lirui Wang[e], Tao Pang[a,c], Yu-Kyoung Oh[f], Chaofeng Zhang[g,**], Hu-Lin Jiang[a,b,c,d,*]

[a] State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China
[b] Jiangsu Key Laboratory of Druggability of Biopharmaceuticals, China Pharmaceutical University, Nanjing 210009, China
[c] Jiangsu Key Laboratory of Drug Screening, China Pharmaceutical University, Nanjing 210009, China
[d] Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, China Pharmaceutical University, Nanjing 210009, China
[e] Jiangsu Key Laboratory of Carcinogenesis and Intervention, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing 210009, China
[f] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Republic of Korea
[g] Research Department of Pharmacognosy, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Keywords:
Vitamin A
Micelle
Chemogene therapy
Liver fibrosis
Silibinin
siRNA

ABSTRACT

Liver fibrosis refers to excessive accumulation of hepatic collagen, which is primarily produced by activated hepatic stellate cells (HSCs). No effective drugs are clinically available to treat this condition, reflecting the fact that antifibrotic drugs do not specifically target activated HSCs. Here, we report the synthesis and evaluation of poly (lactide-*co*-glycolide)-polyspermine-poly (ethylene glycol)-vitamin A (PLGA-PSPE-PEG-VA), and activated HSC-targeted, biocompatible amphiphilic polymers for co-delivery of chemical (silibinin) and genetic (siCol1α1) drugs that synergistically suppress collagen I accumulation in fibrogenesis. PLGA-PSPE-PEG-VA self-assembled into core-shell polymeric micelles (PVMs) at low concentrations. After loading with silibinin and siCol1α1, the resulting chemical/genetic drug-loaded PVMs (CGPVMs) exhibited a small particle size and a slightly positive surface. CGPVMs had very low cytotoxicity and hemolytic activity *in vitro* and were well tolerated in mice, with no liver toxicity or inflammation. Importantly, CGPVMs effectively accumulated in fibrotic livers and specifically targeted activated HSCs. As expected CGPVMs more efficiently decreased collagen I production and ameliorated liver fibrosis compared with chemical drug (silibinin)-loaded PVMs (CPVMs) or genetic drug (siCol1α1)-loaded PVMs (GPVMs) only. These results indicate that CGPVMs are a promising tool for targeted delivery of chemogenes to activated HSCs in the treatment of liver fibrosis.

1. Introduction

Liver fibrosis, a common scarring response to virtually all forms of chronic liver injury, is characterized by activation of hepatic stellate cells (HSCs) and excessive hepatic accumulation of collagen, dominated by collagen type I [1]. The mechanisms underlying the progression of liver fibrosis are fairly well understood. However, no drugs have yet emerged as effective antifibrotic agents. One limiting factor is that medicinal candidates cannot specifically target HSCs and are frequently toxic to parenchymal cells [2–5]. Furthermore, because liver fibrosis is a complicated disease that involves multiple signaling pathways, a single therapeutic agent may have limited antifibrotic effects [3,4,6]. Thus, there is an urgent need to develop specific and effective antifibrotic therapies [2–4,7,8].

Chemogene therapies—combined treatments with chemical and genetic drugs—have been proposed as a strategy for achieving synergistic effects, a concept that has been fully corroborated in cancer therapy [9–12]. However, there are only a few reports of chemogene-delivery systems for the treatment of liver fibrosis [13,14]. Thus, the time may be ripe for the development of an effective chemogene-delivery system for liver fibrosis therapy. Silibinin, a hepatoprotective agent with virtually no toxic side effects, has been widely applied in the clinic to treat various acute and chronic liver diseases, liver fibrosis, and cirrhosis [15,16]. Silibinin has been shown to retard collagen

* Correspondence to: H-L. Jiang, State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China.
** Correspondence to: C. Zhang, Research Department of Pharmacognosy, China Pharmaceutical University, Nanjing 210009, China.
E-mail addresses: zhangchaofeng@cpu.edu.cn (C. Zhang), jianghulin3@163.com (H.-L. Jiang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jconrel.2018.05.032
Received 27 February 2018; Received in revised form 25 April 2018; Accepted 28 May 2018
Available online 29 May 2018
0168-3659/

accumulation in early and advanced fibrosis, with a number of pharmacological studies showing that one of its antifibrotic mechanisms is suppression of the expression of procollagen α1(I) mRNA, encoding the major collagen type in fibrosis [15,16]. However, the reduction in hepatic collagen accumulation by silibinin (~35%) was found to be limited [16], and because of its low specificity, bioavailability and half-life, silibinin can be discounted as a stand-alone liver disease remedy. As a complementary strategy, RNA interference (RNAi) has proven to be a valuable and effective tool for posttranscriptional gene silencing [17]. In this context, small inhibitor RNA (siRNA) targeting Col1α1 (siCol1α1) can be used to specifically block procollagen a1(I) mRNA expression, thereby inhibiting liver fibrosis progression and inducing fibrosis regression [18]. Accordingly, combined delivery of silibinin and siCol1α1 might achieve better remodeling of fibrous collagens than either single agent alone.

Here, we sought to develop a safe system for targeted delivery of chemogene therapy to activated HSCs. First, polyspermine (PSPE), a biocompatible cationic material, was synthesized as previously described [19,20]. Then, PSPE was successfully linked with poly (lactide-*co*-glycolide) (PLGA) and poly (ethylene glycol) (PEG), both of which are Food and Drug Administration (FDA)-approved polymers with good biocompatibility [21–23], forming the amphipathic tri-block polymer, PLGA-PSPE-PEG. Because HSCs have a remarkable capacity for vitamin A uptake, the tri-block polymer was subsequently decorated with vitamin A (VA) to endow our chemogene-delivery system (PLGA-PSPE-PEG-VA) with HSCs specificity [24,25]. PLGA-PSPE-PEG-VA polymers self-assembled into core-shell polymeric micelles (PVMs) and incorporated silibinin into the hydrophobic core of PLGA during the assembly process, leading to the formation of chemical drug-loaded PVMs (CPVMs). Additionally, siCol1α1 was bound to PSPE through electrostatic interactions to form chemical/genetic drug-loaded PVMs (CGPVMs). The various components of these vesicles are designed to contribute specific functionalities to the system. The PEG shell at the periphery of polymeric micelles serves to reduce protein adsorption, prolong circulation time and minimize non-specific uptake, and exposed vitamin A provides specific targeting to activated HSCs. After internalization of polymeric micelles, PSPE buffers the acidic endosome, disrupts membranes by increasing the internal osmotic pressure, and results in endosomal escape of polymeric micelles. Silibinin and siCol1α1 are subsequently released from the polymeric micelles into the cytoplasm and inhibit the expression of collagen I. After successfully synthesizing PVMs, we evaluated their safety and efficiency as a chemogene-delivery system. We further prepared CGPVMs and examined their physicochemical properties. Moreover, we tested the ability of CGPVMs to target HSCs and inhibit collagen I synthesis *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

PLGA (Mw, 20 kDa; lactic acid:glycolic acid, 50:50) with a terminal carboxyl group was purchased from Daigang (Jinan, China). COOH-PEG-COOH (Mw, 5 kDa) was obtained from Xiamen Sinopeg Biotech Co., Ltd. (Xiamen, China). Silibinin, spermine, N, N′-dicyclohexylcarbodiimide (DCC), poly(ethyleneglycol) diacrylate (PEGDA) and branched polyethyleneimine (PEI; Mw, 25 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-hydroxysuccinimide (NHS) was purchased from Aladdin Industries Inc. (Nashville, TN, USA). Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute 1640 (RPMI-1640) medium, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from KeyGEN Biotech (Nanjing, China). Vitamin A was obtained from Energy Chemical (Shanghai, China). Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific (Waltham, USA). Trypsin-EDTA solution (0.25%) was obtained from Gibco (Burlington, Canada). All other chemicals and reagents of the highest purity available were obtained from commercial sources. The following siRNAs were obtained from Shanghai GenePharma Co. Ltd. (Shanghai, China): siCol1α1, 5′-CCU UUC UGC CCA GAA GAA UTT-3′ (sense) and 5′-AUU CUU CUG GGC AGA AAG GTT-3′ (antisense); negative control (scrambled) siRNA, 5′-UUC UCC GAA CGU GUC ACG UdTdT-3′ (sense) and 5′-ACG UGA CAC GUU CGG AGA AdTdT-3′ (antisense). FAM-labeled siRNA (FAM-siRNA) and Cy5-labeled siRNA (Cy5-siRNA) were synthesized by modification of the 5′-end of the sense strand of the scrambled siRNA with FAM or Cy5 (Shanghai GenePharma Co. Ltd.).

2.2. Synthesis and characterization of PLGA-PSPE-PEG-VA

PLGA-PSPE was synthesized as described in our previous study [19]. Briefly, PSPE was synthesized by a Michael addition reaction between PEGDA and spermine; then, PLGA-PSPE was synthesized by a polymerization reaction between the amine group of PSPE and the carboxyl group of PLGA. PLGA-PSPE-PEG-COOH was synthesized using the same method. Briefly, COOH-PEG-COOH (Mw, 5 kDa; 0.5 mmol), DCC (0.6 mmol), and NHS (0.6 mmol) were dissolved in 10 mL of DMSO with stirring at room temperature for 12 h to activate carboxyl groups. DCU was then removed by filtration, and a 10 mL solution of PLGA-PSPE-NH_2 (0.5 mmol) in DMSO was added. The mixture was stirred at room temperature for 24 h and then dialyzed against deionized water (14 K MWCO) for 2 d at 4 °C. After dialysis, PLGA-PSPE-PEG-COOH was lyophilized and stored at −80 °C for later use.

PLGA-PSPE-PEG-VA synthesized by mixing PLGA-PSPE-PEG-COOH (0.05 mmol), DMAP (0.01 mmol), NHS (0.1 mmol), and vitamin A (0.25 mmol) in a 20 mL DMSO solution with stirring in the dark at room temperature for 24 h. The solution was then dialyzed (3.5 k MWCO) for 2 d against DMSO to remove unreacted vitamin A, and then against deionized water for 2 d at 4 °C. After dialysis, the PLGA-PSPE-PEG-VA was lyophilized and stored at −80 °C in the dark for later use. The amount of vitamin A attached to the PLGA-PSPE-PEG was determined by ultraviolet-visible (UV–vis) spectroscopy at 328 nm.

The molecular weights of PSPE, PLGA-PSPE and PLGA-PSPE-PEG were measured by gel-permeation chromatography (GPC) using an LC-20 AB system (Shimadzu, Japan). The column was maintained at a temperature of 40 °C, and 0.05 M sodium sulfate (for PSPE) or a 0.01 M LiBr solution in DMF (for PLGA-PSPE and PLGA-PSPE-PEG) were used as the mobile phase; flow rate was 1 mL/min.

The structures of synthesized polymers were estimated by ^{1}H nuclear magnetic resonance (^{1}H NMR) (Bruker AV-300; Bruker, USA), Fourier-transform infrared spectrometry (FT-IR) (Tenson; Bruker, Germany) and thermal gravimetric analysis (TGA) (NETZSCH TG 209; Netzsch, Germany).

2.3. Measurement of critical micelle concentration

The critical micelle concentration (CMC) of PVMs was measured using the fluorescence pyrene probe technique [10,19]. Briefly, pyrene dissolved in acetone was added to an ampoule bottle, and acetone was removed by volatilization in the dark overnight. Five milliliters of different concentrations of PVMs (0.0001 to 1 g/L) were added to each ampoule bottle containing a final concentration of pyrene of 6×10^{-7} M. After incubating for 24 h at 37 °C in the dark, pyrene fluorescence was monitored at its excitation wavelength and an emission wavelength of 390 nm by fluorescence spectroscopy (Spectra Max M5; Molecular Devices, USA). CMC values were obtained from changes in trends of intensity ratios (I339/I334).

2.4. Measurement of buffering capacity

The buffering capacity of PVMs was measured using acid-base titration. PVMs were dissolved in 10 mL of a 150 mM NaCl solution. The solution was titrated to a starting pH of 10.0 with 0.1 M NaOH using a