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Novel pH-sensitive polysialic acid based polymeric micelles for triggered intracellular release of hydrophobic drug

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ABSTRACT

Polysialic acid (PSA), a non-immunogenic and biodegradable natural polymer, is prone to hydrolysis under endo-lysosomal pH conditions. Here, we synthesized an intracellular pH-sensitive polysialic acid-ursolic acid conjugate by a condensation reaction. To further test the drug loading capability, we prepared paclitaxel-loaded polysialic acid-based amphiphilic copolymer micelle (PTX-loaded-PSAU) by a nanoprecipitation method. Results showed PTX-loaded-PSAU exhibited well-defined spherical shape and homogeneous distribution. The drug-loading was 4.5% with an entrapment efficiency of 67.5%. PTX released from PTX-loaded-PSAU was 15% and 42% in 72 h under simulated physiological condition (pH 7.4) and mild acidic conditions (pH 5.0), respectively. In addition, *In vitro* cytotoxicity assay showed that PTX-loaded-PSAU retained anti-tumor (SGC-7901) activity with a cell viability of 53.8% following 72 h incubation, indicating PTX-loaded-PSAU could efficiently release PTX into the tumor cells. These results indicated that the pH-responsive biodegradable PTX-loaded-PSAU possess superior extracellular stability and intracellular drug release ability.

1. Introduction

Tremendous efforts have been directed to the development of targeted drug delivery systems because they promised to resolve several key therapeutical issues associated with current clinical practice including low treatment efficacy and significant side effects (Langer, 1998; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Until now, various drug delivery systems based on liposomes, nanoparticles, polymeric conjugates and micelles have been intensively explored (Yoo, Lee, & Park, 2002). Polymeric micelles could alter the pharmacokinetic profile of drugs, reduce off-target toxicity and side effects, prolong circulation in the blood owing to its high water-solubility, and enhance the therapeutic efficiency (Cayre, Chagneux, & Biggs, 2011; Kedar, Phutane, Shidhaye, & Kadam, 2010). However, only few drugs could be carried into the intracellular compartments of the cancer cell due to the slow drug release from micelles (Shuai, Ai, Nasongkla, Kim, & Gao, 2004; Sui, Liu, & Shen, 2011). The proton concentration of late endosomes and lysosomes is 100-times lower (pH 5.0) than the physiological condition (pH 7.4), which provide an important stimulus *in vivo* that could be used to trigger intracellular release of hydrophobic drugs (Duncan, 1992; Haag, 2004).

Polysialic acid (PSA), a homopolymer of sialic acid (SA) in either α-2,8 or α-2,9 linkages or a mixture, was first found in *E. coli* (*Escherichia coli*) K-1 and K-235 by Barry (Barry & Goebel, 1957). The 2,8 linked polysialic acid only induced a low level of antibody that could avoid the clearance of modified drugs/carriers from the blood circulation and present long circulation time *in vivo* (Fernandes & Gregoriadis, 1997, 2001). More importantly, it has been proved that PSA is biodegradable and prone to hydrolysis under endo-lysosomal pH conditions (Zhang et al., 2014). Also, the degradation product of PSA, SA, could serve as a targeting ligand for selectin, which is highly expressed in tumor vascular endothelial cells (Greco et al., 2013; Jayant et al., 2007; Zhang et al., 2014). SA could inhibit tumor metastasis by selectin targeting (Zeisig, Stahn, Wenzel, Behrens, & Fichtner, 2004). Obviously, PSA had a great potential to be an effective drug delivery material for targeting cancer disease. Ursolic acid, a natural triterpene, structurally similar to dexamethasone, exhibited antitumor effects in various cell types (Kassi et al., 2007). Research has shown that ursolic acid was relatively non-toxic, and have been used in cosmetics and health products (Liu, 1995).

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In the present work, we reported a novel pH-sensitive degradable micelle for targeted intracellular paclitaxel (PTX) release. Firstly, the amphiphilic copolymer, polysialic acid-ursolic acid conjugate, designed as PSAU was synthesized and characterized. Then, PTX was loaded in PSAU micelle by a nanoprecipitation method. Finally, the particle size, drug entrapment efficiency, drug release behavior *in vitro* and the anti-tumor activity of the PTX-loaded PSAU micelle were investigated.

2. Materials and methods

2.1. Materials

Polysialic acid sodium salt ($M_w = 1.6 \times 10^4$ Da), purchased from Jiangsu Rui Guang Biotechnology Co., Ltd., was further dialyzed against distilled water and lyophilized. Ursolic acid, oxalyl chloride, pyrene, paclitaxel and ethylenediamine were obtained from Aladdin Reagent Int. Bovine serum albumin (BSA) and 3-(4,5-dimethyltiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) were obtained from Sigma–Aldrich Co. All other chemicals and reagents were of analytical grade. The SGC-7901 cell line was a kindly gift from Professor Kan Ding in Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.2. Synthesis of the polysialic acid-based amphiphilic copolymers (PSAU)

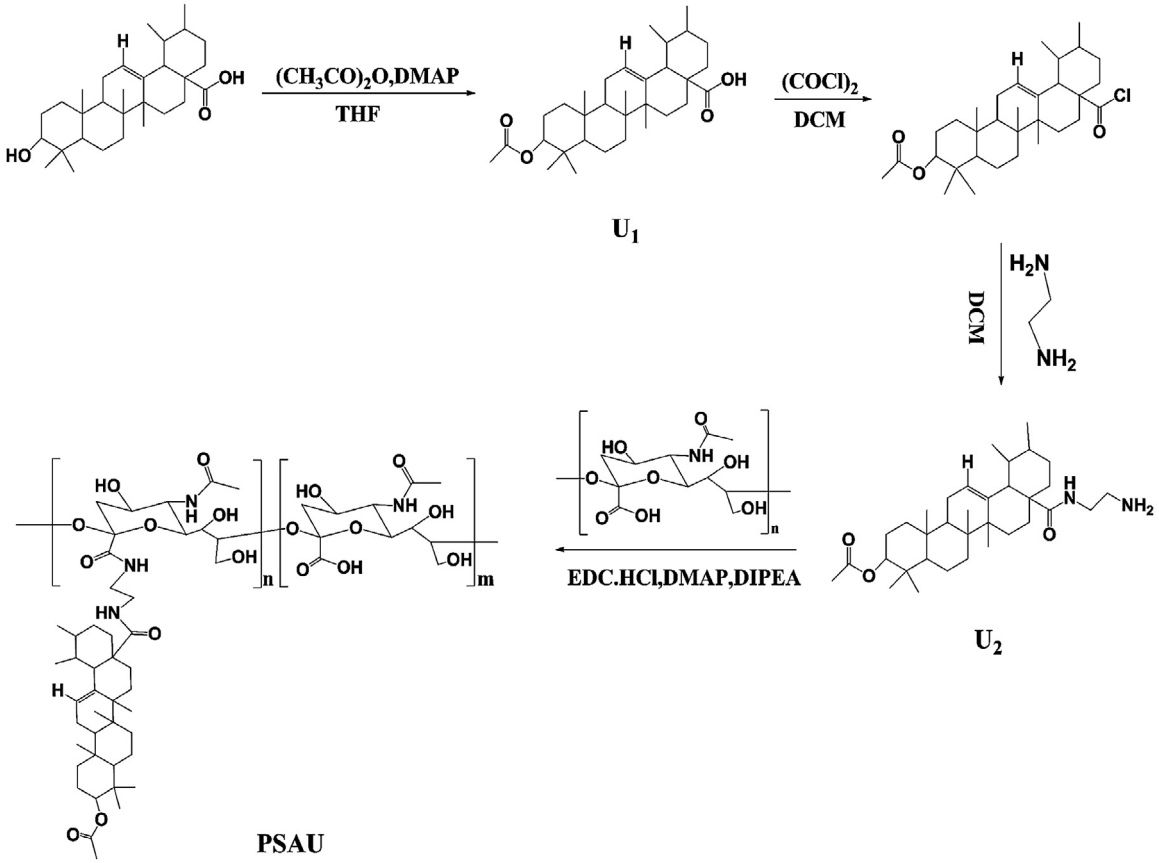
The polysialic acid-based amphiphilic copolymers were synthesized in four reaction steps from PSA, ursolic acid and ethylenediamine (Scheme 1). Specific steps were as follows:

Firstly, 200 mg ursolic acid (0.43 mM) was dissolved in 9 mL anhydrous THF, and 3 mg DMAP (0.025 mM) was added. The resulting solution was cooled by ice bath. Acetic anhydride (600 μL, 6.3 mM) was added dropwise to the solution, and then the mixture was warmed up to room temperature and stirred for another 8 h. The solvent was removed in vacuo and the residual mixture was dissolved with dichloromethane. The solution was washed sequentially with 10% hydrochloric acid, water and brine. Pure U_1 was obtained by silica gel column chromatography.

Secondly, U_1 (0.24 mM) dissolved in 5 mL anhydrous dichloromethane (DCM) was cooled by ice bath. Oxalyl chloride (180 μL, 2.1 mM) was added dropwise. After 30 min, the mixture was warmed up to room temperature and stirred for another 24 h. The solvent was removed in vacuo and the solid was dissolved with anhydrous DCM as a stock solution.

Thirdly, to an ice-cold solution of anhydrous ethylenediamine (12 mM) in 2 mL anhydrous DCM, the stock solution above was added dropwise. After 30 min, the mixture was warmed up to room temperature and stirred for another 12 h. The solution was washed sequentially with 10% hydrochloric acid, water and brine. Aminoethyl U_1 (U_2) was obtained by silica gel column chromatography.

Finally, U_2 (45 mg, 0.083 mM) was dissolved in DMF at 0 °C under N_2, and N,N-diisopropylethylamine (DIPEA, 45 μL, 0.26 mM) was slowly added. The DMF solution was stirred further for 10 min. Polysialic acid (40 mg) was dissolved in 12 mL formamide. DMAP (5.2 mg, 0.043 mM) and EDC·HCl (48 mg, 0.25 mM) were added to the formamide solution within 10 min. The DMF solution and formamide solution were mixed. The mixture was allowed to warm up to room temperature and stirred overnight. The resulting solution was dialyzed against the excess amount of water/methanol (1:3–1:1, v/v) for 1 day and distilled water for another 2 days, respectively. After lyophilization, the polysialic acid-based amphiphilic copolymers (PSAU) were obtained as a white powder.



Scheme 1. Synthetic routes of polysialic acid-based amphiphilic copolymers (PSAU).