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In vivo pharmacokinetics and biodistribution of novel all-trans retinoic acid derivative-loaded, folate-modified poly (L-amino acid) micelles

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ABSTRACT

Folate is widely used as a target ligand for tumor cells, and poly (L-aspartic acid)-*b*-poly-(L-phenylalanine) (PAA-PPA) is a biodegradable material with low immunogenicity and toxicity. In this study, to enhance the targeting effect, folate-conjugated PAA-PPA micelles were synthesized. To evaluate the hypothesis, we assessed the pharmacokinetics and biodistribution of a new antitumor drug 4-amino-2-trifluoromethyl-phenyl retinate (ATPR) loaded into the micelles. We also observed *in vivo* imaging of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) loaded micelles to confirm distribution. The results showed that ATPR-loaded, folate-modified PAA-PPA displayed a prolonged circulation half-life and improved the targeting effect.

1. Introduction

Polymeric micelles are highly appreciated drug delivery systems [1]. They are composed of amphiphilic materials, typically a hydrophilic block and a hydrophobic block [2–4]. Generally, these types of materials can self-aggregate into spherical, nanosized micelles in water [5,6]. Because polymeric micelles are generally about 100 nm, they can be taken up by tumor tissue via an enhanced permeability and retention (EPR) effect [7,8]. These characteristics make polymeric micelles an attractive tumor targeting drug delivery system. Moreover, because the hydrophilic block acts as its outer shell, the micelles show effective resistance to nonspecific uptake by the reticuloendothelial system and an increase in circulation half-life [9]. Additionally, because the inner core is hydrophobic, the micelles can retain poorly water-soluble drugs and significantly improve drug loading. An example of this improved solubility comes from our previous report, showing that polymeric micelles formed from poly (L-aspartic acid)-*b*-poly (L-phenylalanine) (PAA-PPA) improved the solubility of 4-amino-2-trifluoromethyl-phenyl retinate (ATPR) from 0.64 μg/mL to nearly 1 mg/mL [10,11]. Lastly, due to the hydrophobicity of the core, loaded micelles can significantly prevent degradation of poorly water-soluble drugs [10,12].

Though polymeric micelles are widely used as a tumor targeting drug delivery system, the uptake by tumor cells is not optimized. Uptake can be affected by many factors, including the size and/or shape, elasticity, surface charge, surface properties and even cell cycle (we have previously shown that PAA-PPA micelles uptake is cell cycle dependent) [13–18]. Thus, one popular method to improve the targeting efficiency is to modify polymeric micelles with addition of a ligand. Some of the most utilized ligands include RGD peptide [19,20], antibodies [21,22], lactose [23], and folate [24,25]. The folate receptor is a favorable target ligand for increasing uptake due to the heavily increased expression in many human cancer cells. It has been observed that folate receptor conjugation, when attached to nanomatierals, can effectively lead nanoparticles into cancer cells [26,27].

In this study, we synthesized folate-modified poly (L-aspartic acid)-*b*-poly (L-phenylalanine) (FA-PAA-PPA) to assess the uptake efficiency and efficacy as a targeting platform. We investigated *in vivo* pharmacokinetics, biodistribution of FA-PAA-PPA loaded

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with ATPR, and utilized an additional a fluorescent probe-loaded platform to confirm tissue distribution.

2. Materials and methods

2.1. Materials

PAA-PPA and FA-PAA-PPA were synthesized by our lab group as described in a previous study [18] and the synthetic scheme of FA-PAA-PPA is shown in Fig. 1. L-Aspartic acid β-benzyl ester and L-phenylalanine were purchased from Shanghai Hanhong Chemical Co. Ltd. Folate was purchased from Shanghai Yuan Ju Biological Technology Co. Ltd. ATPR (99.73% purity) was kindly provided by Dr. Feihu Chen at the College of Pharmacy of Anhui Medical University [28]. Acetonitrile and methanol were purchased from Tedia Company, Inc. (HPLC grade, Tedia Company, Inc. USA). 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) was purchased from Tianjin Biolite Biotech Co. Ltd. (China). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco Corporation. Fetal bovine serum was purchased from Sijiqing Hangzhou Bioengineering Material Co. Ltd. Penicillin, streptomycin and trypsin were purchased from Beyotime Biotechnology Research Institute. All other reagents were of analytical grade and used without further purification. The HeLa cell line was kindly provided by Dr. Yuxian Shen at the College of Basic Medical Sciences of Anhui Medical University.

2.2. Animals

Sprague-Dawley (SD) rats were supplied by the laboratory animals' center of Anhui Medical University. Specific pathogen-free (SPF) male BALB/cA nude mice (4 weeks old) were obtained from Shanghai Slac Laboratory Animal Co. Ltd. (China).

The animals involved in this study were treated according to the protocols evaluated and approved by the ethical committee of Anhui Medical University (approval number: LLSC 20150297). All the animals were allowed free access to food and water, and experiments were carried out on animals in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

2.3. Preparation of ATPR or DiR loaded micelles and DiR solutions

ATPR loaded micelles were prepared as previously reported [10,11]. Briefly, a solution of ATPR (30 mg in 3 mL DMSO) was added dropwise to a stirring FA-PAA-PPA solution (60 mg of FA-PAA-PPA in 10 mL deionized water) and continued to stir for 20 min, then sonicated for 30 min (JY 92-IID ultrasonic processor, China) in an ice bath. The ATPR loaded FA-PAA-PPA micelles (ATPR-FA-PM) were dialyzed with deionized water at 4 °C for 12 h. Then, ATPR-FA-PM was centrifuged at 3000 rpm for 10 min and filtered with a 0.8 μm pore-sized microporous membrane. Before intravenous (IV) administration to rats, ATPR-FA-PM concentrations were adjusted using normal saline.

Preparation of DiR-loaded micelles was analogous to the method described above using a solution of DiR (1.5 mg of DiR in 0.3 mL DMSO) added dropwise to a stirring solution of PAA-PAA (18 mg of PAA-PPA in 3 mL deionized water) or FA-PAA-PPA (18 mg of FA-PAA-PPA in 3 mL deionized water). DiR loaded PAA-PPA and FA-PAA-PPA micelles were adjusted to the appropriate concentrations with normal saline for caudal vein injection.

2.4. Characterization of ATPR-FA-PM

The Zetasizer Nano ZS90 (Malvern, UK) was used to measure the size and zeta potential of the ATPR-FA-PM. Morphology of the ATPR-FA-PM was assessed with transmission electron microscope (Jeol JEM-2100, Japan) operated at an acceleration voltage of 200 kV, after the solution was negatively stained with 2% (w/v) phosphotungstic acid.

The drug loading percent was calculated as:

Drug loading (%) = (weight of ATPR in micelles)/(weight of ATPR

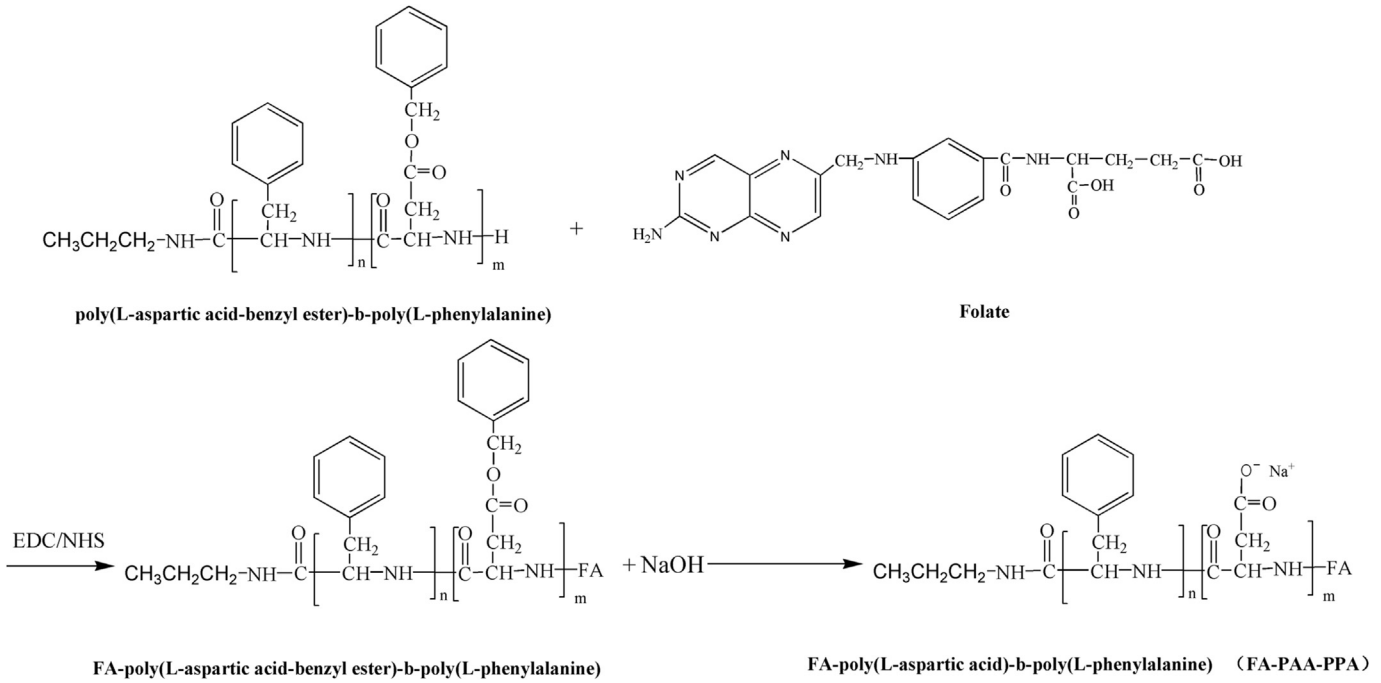

Fig. 1. Synthetic scheme of FA-PAA-PPA.