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Convergent synthesis and characterization of fatty acid-conjugated poly (ethylene glycol)-block-poly(epsilon-caprolactone) nanoparticles for improved drug delivery to the brain

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ABSTRACT

Herein, for the first time, we report the synthesis, preparation, and characterization of fatty acid-conjugated poly (ethylene glycol)-block-poly(epsilon-caprolactone) nanoparticles (FA-NPs), with the ultimate goal of improving drug delivery to the brain. These nanoparticles had a nano-size (< 100 nm) with a fairly narrow size distribution (PDI = 0.2–0.3). They were non-hemolytic and non-cytotoxic. By employing curcumin, a poorly soluble herbal molecule, as a model, FA-NPs enhanced the drug solubility by > 5.4×10^5x. They showed moderate stability in serum and exhibited zero-order drug release. As a proof-of-concept, the brain uptake of oleic acid-NPs (OA-NPs) was examined in rats after intravenous injections. Relative to 1% DMSO and PEG-NPs formulations, the OA-NPs enhanced the brain accumulation of the loaded cargo by 4.28 and 1.94x, respectively. Apparently, these nanoparticles hold considerable promise in drug delivery to the brain and their potential applications for disorders of the central nervous system should be explored.

1. Introduction

Diseases of the central nervous system such as Alzheimer's disease, Parkinson's disease, brain tumors, stroke, etc. are growing threat due to a rapidly growing aging population and a higher life expectancy [1]. Drug delivery to the brain, however, remains an insurmountable challenge owing to two inter-correlated factors: permeability and solubility [2]. The presence of the blood-brain barrier (BBB) greatly reduces the permeation of drug molecules into the brain from the systemic circulation because of the co-existence of tight junctions and various efflux transporters in the BBB. In addition, poor solubility deters therapeutic drug concentrations from being achieved at targets. As a result, ameliorating the BBB permeability in conjunction with solubility enhancement would be highly desirable to improve brain delivery.

Utilizing endogenous transporting systems is an attractive and logical way to improve the BBB permeability [3]. By literature precedent, long chain fatty acids can be efficiently transported across the BBB in humans [4]. It is believed that several groups of fatty acid transporters, such as fatty acid transport protein (1 and 4), fatty acid binding protein (3, 5 and 7) and fatty acid translocase, which are expressed in the human brain microvascular endothelial cells (the major component of the BBB), facilitate the entry of the long chain fatty acids into the brain. However, the applications of fatty acids as brain-targeting ligands have been rarely explored. Fatty acids were often applied to modify protein and peptide-based therapeutics to improve their brain uptake [5]. However, chemical conjugation of fatty acids to drug molecules are often considered as new chemical entities which require a lengthy and costly development process before commercialization. Alternatively, fatty acids can be conjugated to drug carriers for brain targeting.

Fatty acid-conjugated branched polyethylenimine has been prepared to improve gene delivery to the brain [6–8]. However, this system cannot be applied to small drug molecules. Moreover, in these studies, the utilization of fatty acids was mainly for balancing the hydrophobicity of the delivery systems to improve their brain delivery but

Abbreviations: PEG-b-PCL, Poly(ethylene glycol)-block-poly(epsilon-caprolactone); PEG-NPs, PEG-b-PCL nanoparticles; FA-NPs, Fatty acid-conjugated PEG-b-PCL nanoparticles; OA-NPs, Oleic acid-conjugated PEG-b-PCL nanoparticles; C12, Lauric acid; C14, Myristic acid; C16, Palmitic acid; C18, Stearic acid; ALA, Alpha-linolenic acid; LA, Linoleic acid; OA, Oleic acid; DHA, Docosahexaenoic acid; EA, Erucic acid; THF, Tetrahydrofuran; DMF, Dimethylformamide; DMSO, Dimethyl sulfoxide

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the brain-targeting properties of fatty acids has not been fully appreciated [8].

Recent advances in nanotechnology have made possible the development of novel systems for overcoming the drug solubility challenge. Of particular merit in this regard is biodegradable and biocompatible polymeric nanoparticles of poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-b-PCL), which have been extensively applied in drug delivery for the past two decades [9,10]. These amphiphilic polymers form micellar nanoparticles [11], which can efficiently solubilizes poorly soluble drugs and prolongs the circulation time of the loaded drug molecules in the systemic circulation [12].

Apparently, a marriage of fatty acids and PEG-b-PCL nanoparticles can potentially overcome the two major challenges in drug delivery to the brain: poor drug solubility and inefficient delivery across the BBB. Herein, for the first time, we report the synthesis, preparation, characterization and application of fatty acid-conjugated PEG-b-PCL NPs (FA-NPs) for improved drug delivery to the brain. We synthesized a library of fatty acid-conjugated PEG-b-PCL by using a convergent synthetic method. In contrast to traditional linear synthetic strategy, convergent approach enables the incorporation of different fatty acid ligands onto a common intermediate building block. This allows a fair comparison of the influence of different fatty acid ligands on the properties of the nanoparticles without the variability associated with different lots of PEG-b-PCL [13,14]. Aimed at demonstrating the feasibility of improving drug delivery to the brain by FA-NPs, the present study has employed curcumin as a model compound. Curcumin is derived from the rhizome of Curcuma Longa, and it is potentially used to treat neuro-degenerative and neuro-inflammatory diseases, and brain tumors [15,16]. Despite its potential applications in various disease treatments, it suffers from poor solubility and *in vivo* instability, which can be possibly attenuated by using nanotechnology. Accordingly, the objectives of this study were (1) to synthesize and prepare a library of FA-NPs; (2) to fabricate and characterize curcumin-loaded FA-NPs; (3) to examine the *in vitro* toxicity of these nanoparticles; and finally, as a proof-of-concept, (4) to demonstrate the improved drug delivery to the brain in rats.

2. Materials and methods

2.1. Materials

Lauric acid (99.8%), myristic acid (96%), palmitic acid (98%) and stearic acid (99.9%) were purchased from Guangzhou suixin hua gong (Guangzhou, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), erucic acid (90%), ε-caprolactone, 4-dimethylamino pyridine (DMAP) and methanesulfonic acid (MSA) were obtained from J&K Scientific (China). 1-Pentanol, succinic anhydride, oxalyl chloride, α-linolenic acid (> 70%), linoleic acid (> 85%) and tween® 20 were supplied by TCI (Japan). Oleic acid (90%), polyethylene glycol (average M_n = 4600), triethylamine (> 99%), uranyl acetate (98%), pyrene (98%), triton™ X-100, phosphate buffered saline powder (pH 7.4), disodium edetate (EDTA) (> 99%), dodecyl sodium sulfate (SDS) (> 99%) and dimethyl sulfoxide (DMSO) ACS reagent (> 99.9%) were procured from Sigma-Aldrich (St. Louis, MO, USA). *cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6, n-3) (DHA) (98%) and coumarin 6 (99%) were purchased from Santa Cruz Biotechnology (Santan Cruz, CA, USA). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) and Pierce LDH cytotoxicity assay kit were acquired from ThermoFisher Scientific (Hong Kong). Curcumin (> 99.5%) was purchased from Yung Zip Chemical (Dajia, Taiwan). Unless otherwise stated, all materials were obtained from commercial sources and used as received without further purification. All of our synthesis experiments were conducted by using dried glassware and anhydrous solvents in a nitrogen atmosphere. HPLC grade of acetone, acetonitrile and methanol were used as received. AR grade of tetrahydrofuran (THF), toluene, *N,N*-dimethylformamide (DMF), and dichloromethane (DCM) was dried using a 4 Å molecular sieve. Water was purified from Direct-Q® UV Water Purification System (EMD Millipore, USA). Unless otherwise stated, type 1 water was used in the preparation of all aqueous solutions.

2.2. Polymer synthesis

2.2.1. Synthesis of hydroxyl-functionalized PEG-b-PCL, HO-PEG-b-PCL

HO-PEG-b-PCL was synthesized according to a method reported previously [17] using 1-pentanol as an initiator and polyethylene glycol (average M_n = 4600) as a coupling block.

2.2.2. Synthesis of fatty acid conjugated PEG-b-PCL, FA-PEG-b-PCL

A catalytic amount of DMF and oxalyl chloride (10 mmol, 10 equiv.) was added to a solution of a particular fatty acid (1 mmol) in DCM at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After that, it was concentrated *in vacuo* to yield the corresponding fatty acid chloride which was used without further purification. Next, triethylamine (1 mmol) and the fatty acid chloride (1 mmol) were added to a solution of HO-PEG-b-PCL (0.1 mmol) in DCM (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then concentrated *in vacuo* and the residue was dissolved in a minimum amount of DCM and the product was precipitated with methanol. The percentage of the fatty acid conjugation was determined by ^{1}H NMR. Further characterization of the FA-PEG-b-PCL was conducted by using Fourier transform infrared spectrometry (FTIR), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC) and the experimental details of which are summarized in the Supplementary Material.

2.3. Preparation of nanoparticles

Blank and curcumin-loaded nanoparticles were prepared by a nanoprecipitation method [18]. Briefly, various amount of a polymer was dissolved with or without a pre-defined amount of curcumin in 300 μL of a solvent (acetone, acetonitrile, THF, DMF or DMSO. The solution was subsequently added to 1 mL of type 1 water. For the samples prepared with acetone, methanol or THF, they were vortexed for 15 s and placed in a vacuum concentrator for 20 min (45 °C, 0.1 mbar) for the removal of the volatile organic solvent. For the samples prepared with DMF or DMSO, the resulting solutions were dialyzed using Spectra/Por 1 dialysis membrane with an MWCO of 6–8 kD (Spectrum Laboratories, Inc.) against type 1 water for 1 day. After the removal of the organic solvent, the samples were subjected to centrifugation at 16,000*g* for 3 min to remove any un-encapsulated curcumin. The supernatant was collected and used in all our studies and characterization.

2.4. Characterization of nanoparticles

2.4.1. Determination of drug concentration

The concentration of curcumin and coumarin 6 in each formulation was determined by UPLC/UV and the detailed methods are summarized in the Supplementary Material. The drug loading (DL) and encapsulation efficiency (EE) were calculated by the following equations:

$$\mathrm{DL}(\%) = \frac{\text{the weight of the drug in the supernatant}}{\text{the weight of the drug and polymer added}} \times 100\%$$

$$\mathrm{EE}(\%) = \frac{\text{the weight of the drug in the supernatant}}{\text{the weight of the drug added}} \times 100\%$$

2.4.2. Physical characterization of nanoparticles

The cumulative particle size (Z-Average mean particle size, Dz), polydispersity (PDI) and zeta potentials of blank and curcumin-loaded nanoparticles were analyzed by dynamic light scattering (DLS) with