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Synthesis of novel biodegradable and self-assembling methoxy poly(ethylene glycol)–palmitate nanocarrier for curcumin delivery to cancer cells

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Abstract

A novel polymeric amphiphile, mPEG–PA, was synthesized with methoxy poly(ethylene glycol) (mPEG) as the hydrophilic and palmitic acid (PA) as the hydrophobic segment. The conjugate prepared in a single-step reaction showed minimal toxicity on HeLa cells. ^{1}H nuclear magnetic resonance imaging and Fourier transform infrared spectroscopy revealed that the conjugation was through an ester linkage, which is biodegradable. Enzymes having esterase activity, such as lipase, can degrade the conjugate easily, as observed by in vitro studies. mPEG–PA conjugate undergoes self-assembly in an aqueous environment, as evidenced by fluorescence spectroscopic studies with pyrene as a probe. The mPEG–PA conjugate formed micelles in the aqueous solution with critical micelle concentration of 0.12 g l^{-1}. Atomic force microscopy and dynamic light scattering studies showed that the micelles were spherical in shape, with a mean diameter of 41.43 nm. The utility of mPEG–PA to entrap the potent chemopreventive agent curcumin in the core of nanocarrier was investigated. The encapsulation of a highly hydrophobic compound like curcumin in the nanocarrier makes the drug readily soluble in an aqueous system, which can increase the ease of dosing and makes intravenous dosing possible. Drug-loaded micelle nanoparticles showed good stability in physiological condition (pH 7.4), in simulated gastric fluid (pH 1.2) and in simulated intestinal fluid (pH 6.8). This micellar formulation can be used as an enzyme-triggered drug release carrier, as suggested by in vitro enzyme-catalyzed drug release using pure lipase and HeLa cell lysate. The IC_{50} of free curcumin and encapsulated curcumin was found to be 14.32 and 15.58 μM, respectively.

Keywords: Nanocarrier; Curcumin; Anticancer; Micelle; Bioavailability

1. Introduction

Curcumin is a low-molecular-weight natural polyphenol isolated from turmeric (*Curcuma longa*) that has low intrinsic toxicity but a wide range of pharmacological activity, including antitumor, antioxidant, anti-amyloid and anti-inflammatory properties [1]. It is a potent inhibitor of NF-κB, a transcription factor implicated in the pathogenesis of several malignancies [2], and also inhibits the production of various cytokines, including tumor necrosis factor-α and interleukin-1β [3]. Pre-clinical studies of curcumin have shown its ability to inhibit carcinogenesis in a variety of cell lines, including breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic and prostate cancer [4]. As a result, interest is increasing in the clinical development of this compound as a cancer chemopreventive agent [5]. Despite all these promising characteristics, a major problem with curcumin is its extreme low solubility in aqueous solutions, which limits its bioavailability and clinical efficacy [6]. In a clinical study, Shoba et al. showed that after oral administration of 2 g kg^{-1} of curcumin to rats a maximum serum concentration of 1.35 ± 0.23 μg ml^{-1} was observed at time 0.83 h, whereas in humans the same dose of curcumin resulted in

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extremely low (0.006 ± 0.005 μg ml^{-1} at 1 h) serum levels [7]. To increase its aqueous solubility and bioavailability, attempts have been made through encapsulation in liposome, polymeric nanoparticle, lipid-based nanoparticle, biodegradable microsphere, cyclodextrin and hydrogel [8–14]. One possible way of increasing its aqueous solubility is encapsulation within the core of a micellar nanocarrier [15–18].

Interest in nanocarriers for cancer chemotherapy is growing [19]. Polymeric micelles has gained attention as nanocarriers [20–22] due to several advantages, such as (i) their low toxicity; (ii) their high stability; (iii) their small size (<200 nm), which has made them ideal candidate for passive targeting of solid tumor tissue sites by enhanced permeation and retention (EPR) effect [23]; and (iv) they can be prepared in large quantities easily and reproducibly. Several drugs formulated in polymeric micelles are in clinical trial development for the treatment of various cancers [24,25]. Studies on polymeric micelles prepared from block copolymers have been carried out by many groups [26,27], but synthesis of block copolymers by polymerization reaction is a tedious multistep process that requires expertise. Recently many studies have been conducted on self-aggregation behavior of hydrophobically modified water-soluble polymers that can self-assemble to form micelles in the aqueous phase [28,29]. Studies have proved that polymeric amphiphiles consisting of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic low-molecular-weight natural components such as diacyllipid, fatty acid and bile salts can form self-aggregated micelles [30–33].

In this study, we describe a simple method of synthesis and characterization of a novel polymeric amphiphile based on methyl poly(ethylene glycol) (mPEG) as the hydrophilic and palmitate (PA) as the hydrophobic segment. PEG is a well-known biocompatible polymer with an antifouling property and palmitate is a naturally occurring fatty acid in animals. The nonionic hydrophilic PEG shell can suppress opsonin adsorption and subsequent clearance by the mononuclear phagocyte system, thereby prolonging the circulation time, and influences the pharmacokinetics and biodistribution of the drug delivery system [34,35], whereas the hydrophobic core of PA can solubilize curcumin.

The conjugate was synthesized in a single-step reaction. The PEG chain was conjugated with PA through an ester linkage that can be degraded inside the cells. The mPEG–PA conjugate forms self-assembled micelle nanoparticles with hydrophobic anticancer drug curcumin in the core. We also demonstrate enzyme-triggered release of the drug through hydrolysis of the ester linkage of the amphiphilic conjugate by lipase.

2. Materials and methods

2.1. Materials

mPEG (mol. wt. 5000) and palmitoyl chloride was purchased from Fluka (Bangalore, India) and Aldrich (Bangalore, India), respectively. Curcumin was from Himedia Laboratories (Mumbai, India). Porcine pancreatic lipase was from Sigma (Bangalore, India). All the solvents used in the study were of analytical grade and obtained from Merck (Mumbai, India).

Human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (Pune, India). Cells were maintained in Eagle's minimum essential medium, containing 2 mM L-glutamine, 1.5 g l^{-1} sodium bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate, supplemented with 10% fetal bovine serum and 1% antibiotic antimycotic solution (1000 U ml^{-1} penicillin G, 10 mg ml^{-1} streptomycin sulfate, 5 mg ml^{-1} gentamycin and 25 μg ml^{-1} amphotericin B). Cells were cultured at 37 °C in a humidified atmosphere supplied with 5% CO_2.

2.2. Synthesis of mPEG–PA conjugate

The conjugation of mPEG–PA was carried out by reacting mPEG with palmitoyl chloride. mPEG (1 mM) was dissolved in toluene and mixed with triethylamine (final concentration 1 mM). A solution of palmitoyl chloride (1.1 mM) in toluene was added dropwise and stirred continuously for 3 h at 60 °C. The solution was then filtered through filter paper (Whatman, grade 1) to remove the precipitated triethylamine hydrochloride salt. Then mPEG–PA conjugate was precipitated from filtrate by adding cold diethyl ether. The conjugate was characterized by ^{1}H nuclear magnetic resonance imaging (NMR; Mercury Plus 400 MHz, Varian, CA, USA) and Fourier transform infrared (FTIR; Spectrum One, Perkin Elmer, MA, USA) spectroscopy.

2.3. Preparation of self-assembled micellar nanoparticles of mPEG–PA conjugate

The dry powder of mPEG–PA conjugate was dissolved in phosphate-buffered saline (PBS; 0.01 M, pH 7.4) and sonicated for 30 min to get an optically clear solution. The critical micelle concentration (CMC) of mPEG–PA was determined by using pyrene as a hydrophobic fluorescence probe. The CMC was determined based on the intensity of pyrene excitation spectra and shift of the spectra with increasing mPEG–PA concentrations. The pyrene solutions (6×10^{-6} M) in acetone were added to the test tubes and evaporated to remove the solvent. Solutions of mPEG–PA micelles in PBS (0.01 M, pH 7.4) were then added to the test tubes in concentrations ranging from 0.001 to 1 mg ml^{-1}, bringing the final concentration of pyrene to 6.0×10^{-7} M. The solutions were vortexed and kept overnight at 37 °C to equilibrate pyrene with the micelles. Steady-state fluorescence excitation spectra of pyrene were measured at an emission wavelength of 390 nm (Fluoro Max-3, Jobin Yvon, Horiba, USA) with slit widths of 2.5 and 5.0 nm for excitation and emission, respectively.

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