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Polymeric micelles for acyclovir drug delivery

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ABSTRACT

Polymeric prodrug micelles for delivery of acyclovir (ACV) were synthesized. First, ACV was used directly to initiate ring-opening polymerization of ε-caprolactone to form ACV-polycaprolactone (ACV-PCL). Through conjugation of hydrophobic ACV-PCL with hydrophilic methoxy poly(ethylene glycol) (MPEG) or chitosan, polymeric micelles for drug delivery were formed. ^{1}H NMR, FTIR, and gel permeation chromatography were employed to show successful conjugation of MPEG or chitosan to hydrophobic ACV-PCL. Through dynamic light scattering, zeta potential analysis, transmission electron microscopy, and critical micelle concentration (CMC), the synthesized ACV-tagged polymeric micelles were characterized. It was found that the average size of the polymeric micelles was under 200 nm and the CMCs of ACV-PCL-MPEG and ACV-PCL-chitosan were 2.0 mg L^{-1} and 6.6 mg L^{-1}, respectively. The drug release kinetics of ACV was investigated and cytotoxicity assay demonstrates that ACV-tagged polymeric micelles were non-toxic.

1. Introduction

Due to the fact that nanoparticles can be prepared using a variety of polymers, biodegradable polymers have been extensively studied for the field of polymer therapeutics [1–3]. While there have been many biodegradable polymeric nanoparticles synthesized for drug delivery, polymeric micelles have numerous advantages over other proposed colloidal delivery systems [4–7]. Many studies have shown the promise micelles have for drug delivery because they can be tailored for prolonged blood circulation time, cellular selectivity, and for their controlled release capabilities [8–10]. In addition, micelles can be specifically synthesized to increase a drug's solubility and bioavailability [11–14].

Acyclovir (ACV) is a guanosine-based prodrug most commonly used for the treatment of infections caused by herpes simplex virus (HSV) types 1 and 2, varicella zoster virus and, to a lesser extent, cytomegalovirus and Epstein–Barr virus [15]. Moreover, prodrug ACV can be converted to its cytotoxic phosphorylated form by herpes simplex virus thymidine kinase (HSV-TK) gene for cancer therapy [16]. That is, if the HSV-TK gene is delivered to actively dividing cancer cells, and ACV is subsequently administered to the cells, the TK enzyme phosphorylates ACV, yielding toxic metabolites which cause death in prodrug treated HSV-TK expressing cells [17–19]. However, due to ACV's poor water solubility and ensuing low bioavailability, alternative delivery approaches are required to increase the therapeutic potential of ACV. Several methods reported are to couple ACV to biocompatible hydrophilic polymers [20–22] or encapsulation into drug carriers [23–25]. Although these processes increase the bioavailability of ACV as well as offer a practical treatment for patients, they are labor-intensive and cost-ineffective. Recently, we have shown that ACV can be used as an initiator to proceed ring-opening polymerization of ε-caprolactone (ε-CL) to form hydrophobic ACV-polycaprolactone (ACV-PCL) [26], which makes this an economically attractive approach compared with the aforementioned conjugation and encapsulation methods.

Polymeric micelles consist of an inner core made of a hydrophobic block copolymer and an outer corona made of the hydrophilic block of the copolymer. PCL, having been widely used as the core-forming hydrophobic segment of nanoparticles, was selected as the model polymer for this study. PCL is a semi-crystalline, linear resorbable aliphatic polyester. It has been commonly used in drug delivery systems because it is biodegradable and biocompatible [27–29]. PCL is commonly synthesized by ring-opening polymerization of ε-CL using an alcohol as an initiator and stannous (II) octoate ($Sn(Oct)_2$) as a catalyst [30,31]. In addition to using alcohol as the initiator, methoxy-poly(ethylene oxide) and starch have been employed as macroinitiators to form amphiphilic polymers. [32,33]. In this study, prodrug ACV possessing hydroxyl groups was used as the initiator to obtain prodrug-PCL. Then,

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a hydrophilic compound (MPEG or chitosan) was grafted on the hydrophobic prodrug-PCL to form the amphiphilic block copolymer which already has the drug attached started from the ring-opening polymerization. These synthesized ACV-tagged amphiphilic polymers can self-assemble in aqueous medium to form polymeric prodrug micelles for use as nanocarriers in drug delivery.

Individual conjugation of ACV-PCL to a wide array of biocompatible hydrophilic polymers to form polymeric micelles each has their own advantages for drug delivery. With this in mind, we choose to assess the successful conjugation of two model hydrophilic polymers, MPEG and chitosan, to hydrophobic ACV-PCL. Chitosan is a natural polysaccharide derived from deacetylation of chitin. Chitosan's biocompatible and biodegradable features have attracted much attention in biomedical and pharmaceutical research [28,34]. Similarly, MPEG is a biocompatible hydrophilic polymer commonly used in polymeric micelle formation. MPEG is inexpensive, non-toxic and is widely used to covalently modify biological macromolecules and surfaces [10,35,36]. Hence, ACV-PCL-MPEG and ACV-PCL-chitosan copolymers were synthesized. The chemical structure and physical properties of the copolymers were characterized and micelle formation investigated. The drug release profiles of ACV from polymeric prodrug micelles and the biocompatibility of polymeric prodrug micelles were investigated in this study.

2. Experimental

2.1. Materials

ACV was purchased from TCI (Tokyo, Japan). N,N′-dicyclohexyl carbodiimide (DCC), ε-CL, pyrene, and succinic anhydride were purchased from Acros Organics (Geel, Belgium). $Sn(Oct)_2$, $CDCl_3$ with 1% tetramethylsilane (TMS), deuterated dimethyl sulfoxide (DMSO-d_6), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), dichloromethane (DCM), methanol, 2-propanol, hexane, toluene, methoxypolyethylene glycol amine (MPEG-NH_2; MW = 5000), and chitosan oligosaccharide lactate (MW = 5000) were all purchased from Sigma–Aldrich (St. Louis, MO). Ethyl ether was purchased from J.T. Baker (Austin, TX). N-hydroxysuccinimide (NHS) was purchased from Alfa Aesar (Ward Hill, MA). Acetone was purchased from Pharmco-AAPER (Shelbyville, KY). Pyridine and hydrochloric acid (HCl) were purchased from EMD (Philadelphia, PA). Sodium chloride (NaCl) and magnesium sulfate were purchased from Showa (Tokyo, Japan). All reagents were used as received without further purification.

2.2. Characterization methods

Gel permeation chromatography (GPC) analyses were performed on a Waters 1525 binary HPLC pump equipped with a Waters 2414 refractive index detector (Milford, MA). Waters styragel HR 3 (MW = 500–30,000) and HR 4E (MW = 50–100,000) columns were equipped. Molecular weight calibration was performed with polystyrene standards that covered a MW range of 400–4.3×10^4. GPC analyses were performed in THF at a flow rate of 1 mL min^{-1} with an injected volume of 50 μL. ^1H NMR spectra were obtained from a Varian Unity/Inova 400 MHz instrument (Sparta, NJ). To obtain FTIR spectra by a Jasco FTIR-4200 spectrometer (Tokyo, Japan), a small amount of polymeric sample was loaded onto a silicon wafer and THF was added dropwise to dissolve the sample and evaporated afterwards. This was repeated until the entire sample was dissolved and a film had formed.

2.3. Synthesis of ACV-tagged amphiphilic polymers

2.3.1. Synthesis of ACV-PCL

ACV (50 mg) was weighed and mixed with ε-CL (2.25 mL) under a sonication bath for 5 min at room temperature. $Sn(Oct)_2$ (0.5 wt% of ε-CL) was then added into the mixture. The entire solution was placed into a 3-necked round-bottom flask. The system was purged with nitrogen and immersed in an oil bath at 140 °C for 24 h. The crude product was cooled to room temperature, dissolved in DCM, and precipitated by cold methanol. The product was then vacuum dried by a rotary evaporator at 40 °C.

2.3.2. Synthesis of ACV-PCL-COOH

ACV-PCL (0.5 mmol) and succinic anhydride (1 mmol) were weighed and dissolved in toluene in a 3-necked round-bottom flask. One mmol pyridine was added and the solution was reacted under nitrogen at 70 °C for 48 h. The product was then precipitated by cold hexane, and spun down. The pellet was re-dissolved in DCM and washed twice each with 10% (v/v) HCl and saturated NaCl solution. The organic phase was isolated and dried with magnesium sulfate then filtered. The carboxylated ACV-PCL was recovered by precipitation in cold hexane and then vacuum dried by rotary evaporation at 40 °C.

2.3.3. Synthesis of ACV-PCL-NHS

ACV-PCL-COOH (0.54 mmol) and NHS (2.7 mmol) were weighed and mixed in 15 mL DCM, and then DCC (2.7 mmol) was added. The reaction was run under a nitrogen environment at room temperature for 24 h. The precipitated byproduct 1,3-dicyclohexylurea was removed by vacuum filtration. The filtrate was added into 35 mL diethyl ether and cooled to 4 °C for 4 h to precipitate ACV-PCL-NHS. The precipitate was collected by centrifugation at 3500 rpm for 5 min, washed with 2-propanol and solvent removed by rotary evaporation at 40 °C.

2.3.4. Synthesis of ACV-PCL-MPEG

ACV-PCL-NHS (10 mg) and MPEG-NH_2 (10 mg) were weighed and dissolved by 20 mL DCM in a round-bottom flask. The flask was purged with nitrogen and the solution was stirred for 24 h. The solution was then dialyzed (MWCO = 6–8 kDa, Spectra/Por, Rancho Dominguez, CA) against pure DCM to remove remaining MPEG-NH_2. ACV-PCL-MPEG was recovered by rotary evaporation at 40 °C.

2.3.5. Synthesis of ACV-PCL-chitosan

ACV-PCL-NHS (10 mg) was dissolved in 5 mL acetone and slowly added to chitosan solution (20 mg chitosan oligosaccharide lactate dissolved in 25 mL deionized water). The mixture, purged with nitrogen, was stirred in a round-bottom flask for 24 h. The reacted solution was vacuum dried to remove acetone and then lyophilized. The amphiphilic polymer was then dissolved in DCM and dialyzed (MWCO = 6–8 kDa, Spectra/Por) against pure DCM to remove unreacted chitosan. ACV-PCL-chitosan was recovered by rotary evaporation at 40 °C.

2.4. Preparation of polymeric prodrug micelles

ACV-PCL-MPEG and ACV-PCL-chitosan micelles were formed similarly. Briefly, 10 mg of ACV-tagged amphiphilic polymer was dissolved in 2 mL acetone. The solution was then added dropwise to 10 mL deionized water under sonication. Acetone was removed by rotary evaporation and the final solution was collected by filtering through a 0.45 μm filter.

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