Self-Assembled Methoxy Poly(Ethylene Glycol)–Cholesterol Micelles for Hydrophobic Drug Delivery

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ABSTRACT: To promote the application of methoxy poly(ethylene glycol)–cholesterol (mPEG—Chol), mPEG–Chol was used to prepare core–shell micelles encapsulating poorly water-soluble docetaxel (DTX-PM) by modified cosolvent evaporation method. Approaches to enhance DTX entrapment efficiency (EE) and minimize particle size were investigated in detail, including organic and aqueous phase composition, organic/aqueous phase ratio, and polymer concentration. In optimal formulation, micelles had higher EE (97.6%) and drug loading (4.76%) with the diameter of 13.76 ± 0.68 nm and polydispersity index of 0.213 ± 0.006. Transmission electron microscopy (TEM) showed that the micelles were spherical, and differential scanning calorimetry (DSC) analysis proved that DTX was successfully entrapped into mPEG–Chol micelles. The *in vitro* cytotoxicity experiments displayed that blank micelles had no effect on the growth of SKOV-3, BXPC-3, A549, and HepG-2 cells, demonstrating that mPEG–Chol was one of the biocompatible biomaterials. The half inhibition concentration of DTX-PM on SKOV-3, BXPC-3, A549, and HepG-2 cells were 10.08, 7.6, 28.37, and 125.75 ng/mL, respectively. DTX-PM had the similar antitumor activity to free DTX, indicating that mPEG–Chol was a promising micellar vector for hydrophobic drug delivery. In addition, this work provided a new and facile approach to prepare drug-loaded micelles with controllable performances. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1054–1062, 2013

Keywords: cosolvent evaporation; methoxy poly(ethylene glycol); cholesterol; polymers; micelle; docetaxel; stability; hydrophobic drug; calorimetry(DSC); targeted drug delivery

INTRODUCTION

Docetaxel (DTX) is a semisynthetic analogue of paclitaxel, both of which have been used to inhibit the disassembly process of tubulin[1,2] and have shown extraordinary antitumor effect in clinics. As one of the most important cytotoxic agents, DTX has been approved for the treatment of advanced and metastatic breast cancer, non-small cell lung cancer, hormone-refractive prostate cancer, advanced gastric cancer, and so forth.[3,4] Because of DTX's low solubility in water (6–7 μg/mL),[5] its current commercial formulation comprising Tween 80 and 13% ethanol (Taxotere®, Sanofi-Aventis, rue La Boétie, Paris, France) to enhance dissolution has evoked amounts of toxicological side effects, including acute hypersensitivity reaction, accumulative fluid retention, neurotoxicity, musculoskeletal toxicity, neutropenia, and so forth.[6,7] In the past decades, substantial efforts have been focused on various drug delivery systems for these hydrophobic drugs, such as polymeric or inorganic nanoparticles,[8–10] liposomes,[11–13] and micelles,[14–18] to reduce the adverse effects caused by the solvent system and nonspecific delivery of the drug itself. Among these drug delivery systems, polymeric micelles based on biodegradable hydrophilic/hydrophobic polyester have been investigated extensively as their versatility including stability (low CMC values),[16] drug solublization,[19]

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biocompatibility,[20] passive targeting by enhanced permeability and retention effect,[21] and ease of active targeting modification.[15,22,23]

Methoxy poly(ethylene glycol)–cholesterol (mPEG–Chol) with PEG chains linked with endogenous cholesterol[24] can self-assemble to form stable core–shell micelles in water. Moreover, the dense PEG shell of the micelles can avoid protein adsorption and recognition by phagocyte to achieve long circulation *in vivo*. It is also convenient to combine targeting ligands with PEG chains.[25] These advantages confirm it to be a promising carrier material.[26–28] Our group first prepared mPEG–Chol micelles loaded with therapeutic agent (poorly water-soluble drug quercetin) around 120 nm,[29] indicating that other hydrophobic drugs could probably be entrapped by mPEG–Chol micelles with high efficiency. In principle, mPEG—Chol is relatively in small molecular size and has the shorter hydrophobic section Chol as the core of micellar structure, which would lead to a smaller diameter compared with those traditional amphiphilic block copolymer,[30] such as poly (ethylene oxide) (PEO)–poly(ε-caprolactone), PEO–poly(D,L-lactide), and PEO–poly (glycolide).

In this sense, we endeavored to investigate how the formulation variables, particularly procedure parameters in cosolvent evaporation method, would influence the property of mPEG–Chol micelles and its capability of loading DTX. Using single-factor test, we clarified the critical step in this method and made it possible to prepare stable DTX-loaded mPEG–Chol micelles (DTX-PM) around 20 nm. Cell culture assays were performed to assess *in vitro* characterization of DTX-PM. This study provided an extended application of mPEG–Chol in micelles and demonstrated that such a delivery system would be appropriate for hydrophobic drugs encapsulation.

MATERIALS AND METHODS

Materials

Docetaxel (purity 98%) was purchased from Nowa Pharmaceuticals Company, Ltd. (Suzhou, China). mPEG (molecular weight: 2 kDa), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), and dimethylaminopyridine (DMAP) were obtained from Sigma (St. Louis, Missouri) . Chol was obtained from Yuanju Bioscience Technology Company, Ltd. (Shanghai, China). Acetonitrile [high-performance liquid chromatography (HPLC) grade] was supplied by Kermel Chemical Reagent Company, Ltd. (Tianjin, China). All the other chemicals were of analytical grade.

Synthesis and Characterization of mPEG–Chol

Detailed synthetic methods for mPEG–Chol were described in a recent publication.[29] First, the succinyl cholesterol (suc-Chol) was synthesized by the reaction of Chol, DMAP, and succinic anhydride in dichlormethane with a magnetic stirrer. Then, suc-Chol, DMAP, EDCI, and $mPEG_{2000}$ were dissolved in chloroform to react in the same manner. Finally, the composition was recrystallized and dried under vacuum. Both of the two-step reaction were monitored by thin-layer chromatography (TLC), and the product was determined using 400 MHz ^1H NMR (Varian Inc., Palo Alto, California) in $CDCl_3$.

Preparation of DTX-PM

Docetaxel-loaded mPEG–Chol micelles (DTX-PM) were prepared by the cosolvent evaporation method. Briefly, mPEG–Chol and DTX were dissolved in organic solution, which was added dropwise into 10 mL aqueous phase with a magnetic stirrer at room temperature for 20 min. Then, the remained organic phase was rapidly removed in a vacuum at 37°C by rotary evaporation (Rotavapor R-114; Büchi, Flawil, Switzerland). Micelles were finally obtained after the suspension was filtered through a 0.22 μm syringe filter to remove free DTX. Blank micelles without DTX (blank-PM) were prepared by the same method.

In this method, composition of organic and aqueous phase, organic/aqueous phase ratio, and polymers/drug ratio were optimized according to the size, encapsulation, and storage stability of micelles. Except for the observed factor, the others were kept consistent with initial condition: 0.5 mL tetrahydrofuran (THF) contained 20 mg mPEG–Chol and 2 mg DTX as organic phase was added into a constant 10 mL distilled water, and the other procedure followed as given above. First, several general organic solvents with different polarity were investigated including acetonitrile (ACN), THF, methnol (MeOH), ethnol (EtOH), and acetone (DMK). Second, three kinds of isotonic solution [0.9% NaCl, phosphate buffer saline (PBS) 7.4, 5% glucose] were selected to replace distilled water as aqueous phase. Third, the amount of DTX varied from 1 to 6.7 mg while the content of polymer in solution remained 20 mg. Finally, the volume of THF was increased from 0.5 to 3.3 mL. Each batch of experiments was prepared at least in triplicate.

Size and Zeta Potential

The average diameter, particle distribution, and zeta potential of micelles were measured by dynamic light scattering (DLS) on a Zetasizer ZEN3600 particle sizer (Nano-ZS 90 laser particle size analyzer; Malvern Instruments, Malvern, Worcestershire, UK).