Mixed Molecular Weight Copolymer Nanoparticles for the Treatment of Drug-Resistant Tumors: Formulation Development and Cytotoxicity

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ABSTRACT: Nanoparticles composed of both high- and low-molecular-weight methoxy poly(ethylene glycol)-block-poly(caprolactone) (MePEG-b-PCL) diblock copolymers (termed "mixed molecular weight nanoparticles") were investigated for the encapsulation and delivery of the taxane drugs paclitaxel (PTX) and docetaxel (DTX). These nanoparticles were prepared using nanoprecipitation and emulsion methods. These 80 nm nanoparticles were prepared with high yields, efficiently solubilized PTX and DTX up to 500 and 1300 μg/mL, respectively, and demonstrated controlled release of these drugs over 14 days. The taxane-sensitive (MDCKII) and taxane-resistant [P-glycoprotein (P-gp) overexpressing] MDCKII-MDR cell lines were used to establish the cytotoxic profiles of these nanoparticles. Because of the coencapsulation of the previously demonstrated P-gp inhibitor, a low–molecular-weight MePEG-b-PCL copolymer ($MePEG_{17}$-b-PCL_5), these drug-loaded mixed molecular weight nanoparticles dramatically reduced the viability of P-gp overexpressing MDCKII-MDR cells and restored sensitivity to taxane drugs in these cells. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3966–3976, 2014

Keywords: Controlled release/delivery; efflux pumps; multidrug resistance; cancer; P-glycoprotein

INTRODUCTION

The taxanes, paclitaxel (PTX), and docetaxel (DTX) are widely used antineoplastics approved for use in the treatment of a variety of cancers including breast, ovarian, and nonsmall cell lung cancer. Unfortunately, the effective administration of these water-insoluble drugs is hampered by formulation issues whereby the commercial formulations of PTX (Taxol™) and DTX (Taxotere®) contain surfactants that are not well tolerated by patients and offer no controlled release aspect.[1] Furthermore, both PTX and DTX are substrates for P-glycoprotein (P-gp), a transmembrane efflux protein that is overexpressed in multidrug-resistant cancer cells that represents a significant barrier to successful cancer treatment with these drugs.[2]

Nanoparticulate formulations of taxanes may provide a surfactant-free method of solubilizing taxanes and controlled-release properties, and the coencapsulation of a P-gp inhibitor may improve efficacy in drug-resistant tumors. Such nanoparticulate formulations may be administered either systemically by intravenous injection or locally by injection into or close to tumors. Amphiphilic block copolymers composed of a hydrophilic block of methoxy poly(ethylene glycol) (MePEG) covalently bound to a hydrophobic, biodegradable polyester block such as poly(D,L-lactide)[3], poly(caprolactone),[4,5] or poly(lactide-co-glycolide)[6,7] (abbreviated MePEG-b-PDLLA, MePEG-b-PCL, and MePEG-b-PLGA, respectively) have been extensively explored for the delivery of taxanes and other hydrophobic drugs, providing many advantages in the formulation of these compounds. These materials form core–shell nanoparticles that are capable of dramatically enhancing the aqueous solubility of taxanes that may increase the maximum tolerated dose through the use of less toxic materials, thereby improving the safety profile and efficacy compared with the commercial formulations.[8] The highly water bound PEG corona is thought to minimize opsonization, delay capture by the reticuloendothelial system, and increase the circulation time of the nanoparticle promoting accumulation in solid tumors by the enhanced permeation and retention effect. Furthermore, our group has demonstrated that low-molecular-weight MePEG-b-PCL ($MePEG_{17}$-b-PCL_5) is an effective modulator of P-gp resulting in the enhanced uptake of P-gp substrates including PTX.[9–13]

Most studies investigating the delivery of taxanes by block copolymer nanoparticles have focused on the strategy of drug solubilization for systemic administration with prolonged circulation times for improved tumor uptake after intravenous administration.[14] However, we have recently demonstrated that taxane-loaded nanoparticles offer another potentially important delivery strategy, based on our finding that the intracellular accumulation of drug and subsequent cytotoxicity were enhanced in both drug-sensitive and drug-resistant (P-gp overexpressing) cells following the application of ultrasound to the cells.[15] These findings suggest a role for these nanoparticles whereby local delivery may be followed by focused ultrasound administration. We suggest that if stable, taxane-loaded nanoparticles were taken up into cells to form a controlled-release intracellular depot of drug, then coencapsulation of the P-gp inhibitor, $MePEG_{17}$-b-PCL_5, within the nanoparticle may also inhibit drug efflux from drug-resistant cells and enhance cytotoxicity.

Using a similar strategy but without ultrasound, doxorubicin has been coformulated in a variety of nanoparticles with a number of P-gp inhibitors including verapamil, cyclosporine, siRNA, and G918 and thus allowing for greater doxorubicin

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accumulation in drug-resistant cells.[16–18] Similarly, PTX encapsulated in lipid-based nanoparticles or formulated as nanocrystals, both including surfactant-type P-gp inhibitors, have been shown to reduce the mass of drug-resistant tumors *in vivo* by a mechanism proposed to include endocytosis of the nanoparticles and likely the intracellular release of the surfactant after nanoparticulate uptake.[19,20] Furthermore, a number of taxane-loaded Pluronic-containing mixed nanoparticle systems have been investigated for the treatment of drug-resistant cancers.[21–25] Because of a well-documented ability to inhibit P-gp, Pluronic systems have been demonstrated to significantly decrease the IC_{50} of the encapsulated drug, as compared with free drug or the commercial formulation. However, in some cases, the carrier itself was shown to be cytotoxic at relatively low-copolymer concentrations, and in most reports, the viability of the drug-resistant cells remained relatively high despite treatment with high concentrations of drug.[23,24,26,27]

In this work, we developed a mixed molecular weight nanoparticulate system called mixed MW PCL_{200}/PCL_5 nanoparticles that are composed entirely of biodegradable and biocompatible MePEG-b-PCL. A high-molecular-weight diblock copolymer, $MePEG_{114}$-b-PCL_{200}, was used to maximize taxane loading and stability, and a low-molecular-weight $MePEG_{17}$-b-PCL_5 was used as a P-gp inhibitor. We report on the *in vitro* physicochemical characteristics and cytotoxicity of these nanoparticles loaded with PTX and DTX. We demonstrated profound cytotoxic effects of these nanoparticles on a MDR cell line that were markedly superior to previously reported Pluronic-mixed micelles using similar or lower drug concentrations.

MATERIAL AND METHODS

Materials

ε-Caprolactone was purchased from Alfa Aesar (Ward Hill, Massachusetts). Stannous octoate and MePEG were obtained from Sigma–Aldrich Canada Ltd. (Oakville, Ontario, Canada), PTX from Polymed Therapeutics Inc. (Houston, Texas), DTX from Natural Pharma (Vancouver, British Columbia, Canada), ^{3}H PTX and ^{3}H DTX from Moravek Biochemicals Inc. (Brea, California), and Dulbecco's modified Eagle's media supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin and Hank's buffered salt solution (HBSS) from Invitrogen (Grand Island, New York). The solvents chloroform, ethyl acetate, anhydrous toluene, hexane, diethyl ether, acetonitrile, methanol, N, and N-dimethylformamide were all purchased from Fisher Scientific Company (Ottawa, Ontario, Canada). Deuterated chloroform was obtained from Cambridge Isotope Laboratories (Andover, Massachusetts). The MTS cell proliferation assay kit and BCA protein assay kit were purchased from Promega (Madison, Wisconsin) and Thermo Scientific (Rockford, Illinois), respectively. MDCKII and MDCKII-MDR cells were kind gifts from Dr. Piet Borst (National Cancer Institute, The Netherlands).

Synthesis and Characterization of Copolymers

A high-molecular-weight MePEG-b-PCL copolymer, termed $MePEG_{114}$-b-PCL_{200}, was synthesized by reacting MePEG (MW 5000 g/mol) with ε-caprolactone in a weight ratio of 18:82. The copolymer was synthesized in anhydrous toluene under a nitrogen atmosphere at 110°C for 19 h using stannous octoate as a catalyst. The toluene was removed under vacuum after completion of the reaction. A low-molecular-weight MePEG-b-PCL copolymer, termed $MePEG_{17}$-b-PCL_5, was synthesized as previously reported by reacting MePEG (MW 750 g/mol) with ε-caprolactone in a weight ratio of 60:40 also using stannous octoate as a catalyst.[10,28] The reaction was allowed to proceed for 4 h at 140°C with stirring under a nitrogen atmosphere. The products were purified by dissolving the copolymers in chloroform followed by precipitation with a 70/30 mix of hexane and diethyl ether. The block lengths of the copolymers were determined by 1D ^{1}H nuclear magnetic resonance (NMR) using a 400 MHz Bruker Advance II+ spectrometer (Bruker Corporation, Milton, Ontario, Canada). The degree of polymerization (DPn) was determined using the peaks situated around 1.4 and 1.66 ppm, attributed to the caprolactone methylene protons, and the peaks at 3.65 ppm, assigned to the MePEG methylene protons. The molecular weights and molecular weight polydispersity indexes of the copolymers were determined using gel permeation chromatography (GPC) with polyethylene glycol standards in the range of 400–22,800 g/mol (Polymer Laboratories Inc., Amherst, Massachusetts) for the low-molecular weight copolymer or polystyrene standards in the range of 4000–100,000 g/mol (Polysciences Inc., Warrington, Pennsylvania) for the high-molecular-weight copolymer. Briefly, samples were injected using a Waters (Milford, Massachusetts) model 717 plus autosampler and separation was achieved through a Waters Styragel HR 3. Chloroform with a flow rate of 1 mL/min was used as the mobile phase. Detection was through a Waters model 2410 refractive index detector.

Preparation and Characterization of Nanoparticles

Nanoparticles were prepared by an oil-in-water emulsion technique or a nanoprecipitation and dialysis method. In the emulsification method, $MePEG_{114}$-b-PCL_{200} was dissolved in 500 μL of ethyl acetate at a concentration of 100 mg/mL with a specified amount of $MePEG_{17}$-b-PCL_5 (ranging from 0 to 100 mg/mL) to stabilize the emulsion. The oil phase was emulsified in 5 mL of aqueous buffer [10 mM phosphate-buffered saline (PBS) for drug loading and release studies or HBSS for cell studies] using a 100 W tip sonicator (Sonic Dismembrator; Fisher Scientific Company) for 1 min in an ice bath. The nanoparticulate dispersions were stirred overnight to allow the ethyl acetate to evaporate. The dispersions were diluted to a final volume of 5 mL with the appropriate buffer and then centrifuged at 11952 g for 5 min to remove any precipitate prior to further studies. The final concentration of $MePEG_{114}$-b-PCL_{200} in the dispersion was 1% (w/v) with the $MePEG_{17}$-b-PCL_5 concentration ranging from 0% to 1% (w/v). The hydrodynamic diameter and zeta potential of nanoparticles was determined using a Malvern Zetasizer Nano ZS (Malvern, Westborough, Massachusetts). Nanoparticles were also prepared in the absence of $MePEG_{17}$-b-PCL_5 using a nanoprecipitation and dialysis technique and are referred to as PCL_{200} nanoparticles. In this method, 30 mg of $MePEG_{114}$-b-PCL_{200} was dissolved in 1 mL of dimethyl formamide (DMF) and this was added dropwise to 1 mL of rapidly stirring PBS or HBSS (for cell studies). This dispersion was then transferred to a 3500 MWCO dialysis tube and dialyzed against 4 L of buffer overnight. The final nanoparticle dispersion was made up to 3 mL with the appropriate buffer. These nanoparticle suspensions were found to be stable for many days on the bench without any evidence of drug precipitation.