International Journal of Pharmaceutics 510 (2016) 365–374

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Multidrug PLA-PEG filomicelles for concurrent delivery of anticancer drugs—The influence of drug-drug and drug-polymer interactions on drug loading and release properties

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ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form 17 June 2016
Accepted 20 June 2016
Available online 23 June 2016

Keywords:
Combination therapy
Multidrug micelles
PLA-PEG
Paclitaxel
17-AAG
Rapamycin

ABSTRACT

This study aimed to analyze the influence of drug-drug and drug-polymer interactions on drug loading and release properties of multidrug micelles. Three hydrophobic drugs—paclitaxel (Ptx), 17-AAG and rapamycin (Rap) were incorporated in poly(L-lactide)-poly(ethylene glycol) (PLA-PEG) filomicelles. Double loaded micelles containing Ptx and 17-AAG were used for the sake of comparison. ^{1}H NMR confirmed the effective incorporation of the various drugs in micelles, and HPLC allowed to determine the drug loading contents. FTIR was used to evaluate interactions between particular drugs and between drugs and copolymer.

Ptx and 17-AAG present similar loading efficiencies in double loaded micelles probably due to interactions of drugs with each other and also with the copolymer. In contrast, unequal drug loading properties are observed for triple loaded micelles. Rapamycin shows very weak interactions with the copolymer, and displays the lowest loading efficiency.

In vitro release of drugs from micelles was realized in pH 7.4 phosphate buffered saline at 37 °C, and monitored by HPLC. Similar release profiles are observed for the three drugs: a strong burst followed by slower release. Nevertheless, Ptx release from micelles is significantly slower as compared to 17-AAG and Rap, probably due to interactions of NH and OH groups of Ptx with the carbonyl group of PLA.

In vitro cytotoxicity of Ptx/17-AAG/Rap loaded micelles and a mixture of free drugs was determined. Drug loaded micelles exhibit advantageous effect of prolonged drug release and cytotoxic activity against Caco-2 cells, which makes them a promising solution for simultaneous drug delivery to solid tumors. Therefore, understanding of interactions within multidrug micelles should be a valuable approach for the development of concurrent delivery systems of anticancer drugs with tailored properties.

1. Introduction

Combination therapy is described as simultaneous administration of two or more pharmacologically active agents with different mechanisms of action, and is recognized as more efficient compared to conventional therapy based on a single therapeutic agent (Aw et al., 2013; Cho et al., 2015). Drug combination in anticancer treatment primarily aims to overcome tumor heterogeneity and multidrug resistance (MDR), and to achieve additive or more desirable synergistic anticancer efficacy without overlapping toxicity (Aw et al., 2013; Mi et al., 2013). Currently, multiple drug delivery is only realized by sequential intravenous infusion with use of excipients such as Cremophor EL in Taxol® (Aw et al., 2013). But these excipients often cause hypersensitive reactions, *e.g.* severe hypersensitivity reactions in >30% patients, neuro- and nephrotoxicity (Aw et al., 2013; Gelderblom et al., 2001). Moreover, Cremophor EL could interact with the components of poly(vinyl chloride) bags and infusion lines, thus causing toxicity (Narvekar et al., 2014). Therefore, a single carrier for several drugs should allow to maximize cytotoxicity to targeted cells while minimizing cell resistance to any of them (Ahmed et al., 2006).

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http://dx.doi.org/10.1016/j.ijpharm.2016.06.051
0378-5173/

Recently, polymeric micelles, in particular those prepared from polylactide-poly(ethylene glycol) (PLA-PEG) block copolymers, have been extensively studied as drug carrier because of many advantageous properties including bioresorbability, controlled drug release, ability to avoid reticuloendothelial system (RES) uptake, tumor targeting by enhanced permeability and retention (EPR) effect, *etc.* (Mu et al., 2010). Micelles exhibit a core-shell structure which allows to encapsulate drugs of poor water solubility in the hydrophobic core, the outer hydrophilic corona providing biocompatibility and prolonged circulation in the bloodstream by avoiding rapid clearance by the liver and spleen. Thus, polymeric micelles present great interest for concurrent multidrug delivery because of simplified administration, improved safety, and simultaneous and possible synergistic action of multiple drugs at solid tumors (Cho et al., 2013; Hasenstein et al., 2012; Shin et al., 2011; Shin et al., 2012).

Co-incorporation of two or three hydrophobic drugs in micelles has already been reported (Aw et al., 2013; Katragadda et al., 2013; Katragadda et al., 2011). PLA-PEG micelles containing paclitaxel (Ptx), 17-AAG and rapamycin (Rap) known as Triolimus present a 10–10,000-fold increase in aqueous solubility of drugs, and display potent cytotoxic synergy *in vitro* and antitumor activity *in vivo* (Cho et al., 2015; Hasenstein et al., 2012). Paclitaxel acts as a potent inhibitor of cell replication by binding to β-subunit of tubulin, enhancement of tubulin polymerization, and also interaction with microtubules. These effects stabilize the microtubules against depolymerization of calcium ions and inhibit the interphase, mitotic cellular functions, and apoptosis (Reddy and Bazile, 2014; Xie and Wang, 2005). Rapamycin may increase and prolong the sensitivity to microtubule directed chemotherapeutic agents (Mondesire et al., 2004; VanderWeele et al., 2004), and exert independent activity in some settings (Wu and Hu, 2010). The third agent, 17-AAG (17-allylamino-17-demethoxygeldamycin) also called tanespimycin, is a heat shock protein 90 (HSP90) inhibitor. It possesses independent activity in several settings, and is able to target compensatory pathways activated by mTOR inhibition, including Akt activation (Hasenstein et al., 2012). 17-AAG in combination with rapamycin presents synergistic activity on multiple myeloma (MM) cells *in vitro* (Francis et al., 2006). Moreover, 17-AAG sensitizes cancer cells to apoptosis induced by paclitaxel when both drugs are simultaneously administered or when 17-AAG treatment is followed by paclitaxel (Munster et al., 2001; Nguyen et al., 1999).

It is generally recognized that physical encapsulation of hydrophobic drugs in micelles is mainly driven by interactions between drug and hydrophobic segments of polymers (Ma et al., 2015). Incorporation of more than one drug makes the system more complex because interactions between drugs may occur apart from drug-polymer interactions. Nevertheless, the influence of drug-drug and drug- polymer interactions on drug loading and release properties has not been investigated, so far.

In our previous work, prolonged paclitaxel release was obtained from PLA-PEG filomicelles under physiological conditions (Jelonek et al., 2015). Flexible worm-like "filomicelles" can be up to 8 μm long and in analogy to filoviruses—possess a long circulation time up to a week in the bloodstream because their unique visco-elastic properties and hydrodynamics could reduce interactions with the blood vessel walls (Cai et al., 2007; Loverde et al., 2011; Venkataraman et al., 2011). The drug release rate was found to be mainly dependent on the degradation of micelles. The slow and prolonged drug release is very advantageous taking into account that only 10–15% of tumor cells are expected to be in the mitotic phase of cell division at any time (Wolinsky et al., 2012). In the present study, PLA-PEG filomicelles as multiple drug carrier were investigated to elucidate the influence of drug-drug and drug-polymer interactions on drug loading and drug release properties. The outcomes of this work should be very helpful for the development of novel multiple drug delivery systems in cancer treatment.

2. Materials and methods

2.1. Materials

PLA-PEG diblock copolymer was synthesized according to the procedure described before (Jelonek et al., 2015). The M_n of PLA and PEG blocks was 6100 and 5000, respectively. Paclitaxel, 17-AAG and rapamycin were purchased from LC Laboratories (Woburn, MA, USA). Caco-2 human colorectal adenocarcinoma cell line was obtained from American Type Culture Collection (ATCC-LGC Standard). Fetal bovine serum (FBS) was purchased from PAN-Biotech. All other organic solvents were of analytic grade from Sigma-Aldrich and used without further purification.

2.2. Preparation of micelles

Drug-free micelles were prepared by using nanoprecipitation method. Briefly, the copolymer was dissolved in chloroform and mixed with distilled water to obtain a concentration of 1 mg/mL. The mixture was stirred vigorously at room temperature for 4 h and left for solvent evaporation for 24 h, followed by filtration through 0.85 μm filter. Drug loading in micelles was conducted by co-solvent/evaporation method. Two drug combinations were prepared: Ptx/17-AAG/Rap and Ptx/17-AAG. The ratio of drugs to polymer (w/w) was 10% or 25% and each drug was used in equal amount. The drugs were dissolved in methanol, and the solution was added to a micellar solution. The mixture was stirred vigorously for 5 h, followed by centrifugation at 3000 rpm for 5 min to eliminate unloaded drugs. The supernatant was recovered, lyophilized and stored at 4 °C for further analysis.

2.3. Characterization of PLA/PEG micelles

The morphology of micelles was analyzed using multimode atomic force microscopy (AFM) instrument (MultiMode, di-Veeco, USA, CA) with NanoScope 3D operating in the tapping mode in air with standard 125 μm single-crystal silicon cantilevers (Model TESP; Bruker; USA, CA). A 1.0 g/L micellar solution was 20 times diluted. A drop of the diluted solution was deposited on a mica disc, spin-coated at 120 rpm and air dried overnight. Measurements were performed at room temperature. WSxM (Nanotec Electronica) software package was used for image processing.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was performed on Bruker-Avance II Ultrashield Plus spectrometer operating at 600 MHz. Spectra were obtained with 32 scans, 11 μs pulse width, and 2.65 s acquisition time. $CDCl_3$ was used as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference.

Fourier transform infrared (FTIR) spectra were recorded on BioRad FTS-40 spectrometer. 32 scans were accumulated at 2 cm^{-1} resolution in the region between 4000 and 400 cm^{-1}. Samples were prepared by depositing a drop of chloroform solution onto a potassium bromide plate, followed by solvent evaporation.

2.4. Quantification of drug loading content

Quantitative assessment of paclitaxel, 17-AAG and rapamycin was conducted by means of reverse phase high performance liquid chromatography (RP-HPLC) using a VWR/Hitachi LaChrom Elite® chromatograph. A Zorbax SB-C8 column (4.6 × 75 mm, Agilent Technologies) was used for the separation of the analytes. The mobile phase was a mixture of 45/55 acetonitrile/water containing