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Novel pH-sensitive charge-reversal cell penetrating peptide conjugated PEG-PLA micelles for docetaxel delivery: In vitro study

Ammar Ouahab, Nihad Cheraga, Vitus Onoja, Yan Shen **, Jiasheng Tu *

Department of Pharmaceutics, China pharmaceutical University, #24 Tongjiaxiang, Nanjing 210009, China.

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ABSTRACT

In order to create a pH-sensitive charge-reversal system for cell penetrating peptides (CPP) to prevent non-specific internalization of the drug; and concomitantly enhance the physical stability and tumor targetability of poly(ethylene glycol)-poly(D,L-lactide) (PEG-PLA) micelles, two sets of novel PEG-PLA micelles were developed. Cell penetrating decapeptide arginine-glycine $(RG)_5$ and a pH-sensitive masking decapeptide histidine-glutamic acid $(HE)_5$ were conjugated at the PEG free end to produce pH sensitive with peptides outside micelles (PHPO), while the pH sensitive with peptides inside micelles (PHPI) are the micelles obtained with the two peptides conjugated to the free end of the PLA block. The polymers were successfully synthesized and characterized by ^{1}H NMR and GPC. The mixed micelles were prepared and characterized for their loading efficiency, particle size and zeta potential. The surface charge of PHPO was greatly affected by the pH of the solution and $(RG)_5$:$(HE)_5$ ratio at the surface. The pH value of the solution at which the surface charge of PHPO reversed could be manipulated by the feed ratio of $(RG)_5$-PEG-PLA (RGO) and $(HE)_5$-PEG-PLA (HEO), hence, HEO:RGO molar ratio of 45:55 was selected for tumor targeting. Docetaxel (DTX) was sufficiently solubilized by DTX-PHPO with a loading efficiency of $90.18 \pm 1.65\%$. At pH 7.4, DTX loaded mPEG-PLA (DTX-PM) (41.2 ± 0.3 nm), DTX-PHPO (195.3 ± 1.9 nm) and DTX-PHPI (190.9 ± 4.5 nm) showed sustained DTX release of less than 55% within 48 h. However, at pH 6.8 DTX-PHPI released $87.29 \pm 0.24\%$, while DTX-PHPO released $70.49 \pm 0.39\%$ of the initial DTX amount within 48 h. Moreover, the physical stability of DTX-PHPO was increased due to the electrostatic interaction of the two peptides. The cellular uptake of DTX-PHPO in SGC-7901 cells and the cell killing effect tested on MCF-7 cells were enhanced by 2 folds at pH 6.8 compared to pH 7.4. Hence, DTX-PHPO is highly pH-sensitive in mildly acidic pH and exhibited higher internalization, but DTX-PHPI exhibited accelerated release. Meanwhile, both formulations displayed low internalization and release at pH greater than 7. This pH sensitive charge reversal design can offer a promising safe carrier using both CPPs and PEG-PLA micelles.

1. Introduction

Docetaxel (DTX) is of the chemotherapy drug class; taxane, and is a semi-synthetic analog of paclitaxel, an extract from the bark of the rare Pacific yew tree "*Taxus brevifolia*" (Genevieve et al., 2010), it is used against advanced and metastatic breast cancer, non-small cell lung cancer, and advanced gastric cancer (Baker et al., 2009). However, non-ionic toxic surfactants such as Tween 80 are being used to dissolve DTX in its current commercial formulation (Baker et al., 2009). Polymeric micelles have been studied extensively as surfactant-free delivery systems for injectable drug formulations of poorly water-soluble drugs (Donghua et al., 2010; Rapoport, 2004; Shuai et al., 2004), but since DTX distributes throughout the body in a nonspecific manner; active targeting using specific ligands was applied to deliver the drug into the desired site (Allen, 2002; Ling et al., 2011; Rijcken et al., 2007; Schottelius et al., 2009). Furthermore, introducing stimuli-sensitive segments in the block copolymer generates polymeric micelles with controlled release mechanisms (Cheng et al., 2013; Nitta and Numata, 2013). In order to improve PEG-PLA polymeric micelle stability and targeting effect, interest in its modification has meant much research being done on its various chemical reactions (Kataoka et al., 2001). Some of the modifications are the stabilization of the micelles by ionic interaction either on the shell or in the core (Eun et al., 2008; Eun et al., 2005) and the conjugation of targeting ligands on the surface (Lee et al., 2003; Wang et al., 2007; Uttam et al., 2010).

Cell penetrating peptides (CPPs) are short peptides that are able to accumulate in the cytoplasm through endocytosis-dependent mechanism (Patel et al., 2007; Richard et al., 2005) and ferry cargo

* Corresponding author. Tel.: +86 25 83271305, fax: +86 25 83301606.
** Corresponding author. Tel.: +86 25 83271305/13815416429.
E-mail addresses: wahab_am@hotmail.fr (A. Ouahab), shenyan19820801@126.com (Y. Shen), jiashengtu@yahoo.com.cn (J. Tu).

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molecules into the cells, including large particles (Patel et al., 2009; Tréhin and Merkle, 2004). They are usually short peptides of less than 30 amino acids and their penetration into cells is rapid and of first-order, with half-times from 5 to 20 min (Son et al., 2007). For instance, arginine-glycine $(RG)_5$ oligopeptide exhibit unique internalization properties. However, their application in drug delivery is limited due to their lack of specificity. At neutral pH, $(RG)_5$ and histidine-glutamic acid $(HE)_5$ oligopeptides present net charges of +5 and −4.5, respectively (PepCalc.com, 2013; http://pepcalc.com/peptide-solubility-calculator.php), and they both present cell penetrating capacities (Rothbard et al., 2002; Likun et al., 2011). Incorporation of histidine residues in the polymer induces higher internalization at pH 6 than pH 7.4 (Liu et al., 2011; Lo and Wang, 2008; Zhang et al., 2011). The imidazole group of histidine ($pK_a = 5.97$) in $(HE)_5$ is neutral at physiological pH 7.4, allowing for the electrostatic interaction between anionic γ-carboxylate groups of glutamic acid ($pK_a = 4.25$) and cationic ε-amino groups of guanidino groups of arginine ($pK_a = 13.2$). After exposure to mildly acidic pH, the imidazole group in histidine gradually changes from neutral to cationic, thus the cell penetrating activity of $(RG)_5$ will be gradually regenerated at mildly-acidic pH. This regeneration process is similar to the dissociation between HE-oligopeptides and CPPs in recombinant peptide constructs reported recently (Jennica et al., 2012). The hypothetical model of these mixed polymeric micelles and their functions are illustrated in Fig. 1.

In the present work, $(RG)_5$ and $(HE)_5$ were conjugated to the COOH terminus of COOH—PEG-PLA to produce $(RG)_5$-PEG-PLA (RGO) and $(HE)_5$-PEG-PLA (HEO) polymers, and to mPEG-PLA—COOH to produce mPEG-PLA-$(RG)_5$ (RGI) and mPEG-PLA-$(HE)_5$ (HEI) polymers. Mixed micelles of RGO and HEO at different ratios were prepared to create stabilized pH sensitive charge reversal CPP conjugated on the surface of polymeric micelles (PHPO), while RGI and HEI were mixed to prepare stabilized pH sensitive peptides conjugated in the core of polymeric micelles (PHPI). The prepared DTX loaded mixed micelles were characterized for their drug loading, particle size, in vitro release profile, cytotoxicity, and cellular uptake.

2. Materials and methods

2.1. Materials

Poly (ethylene glycol) (HO—PEG—OH) and methoxy-poly (ethylene glycol) (mPEG—OH) with M_w of 2000 g/mol were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). D,L-Lactide was purchased from Jinan Daigang Biotech Co., Ltd. (Batch No: 20080826, Jinan, China). Succinic anhydride was obtained from Sigma–Aldrich (Lot No: MKBG0885V, Missouri, USA). Histidine-glutamic acid $(HE)_5$ oligopeptide (95% purity, Batch No: 12111792) and arginine–glycine $(RG)_5$ oligopeptide (95% purity,

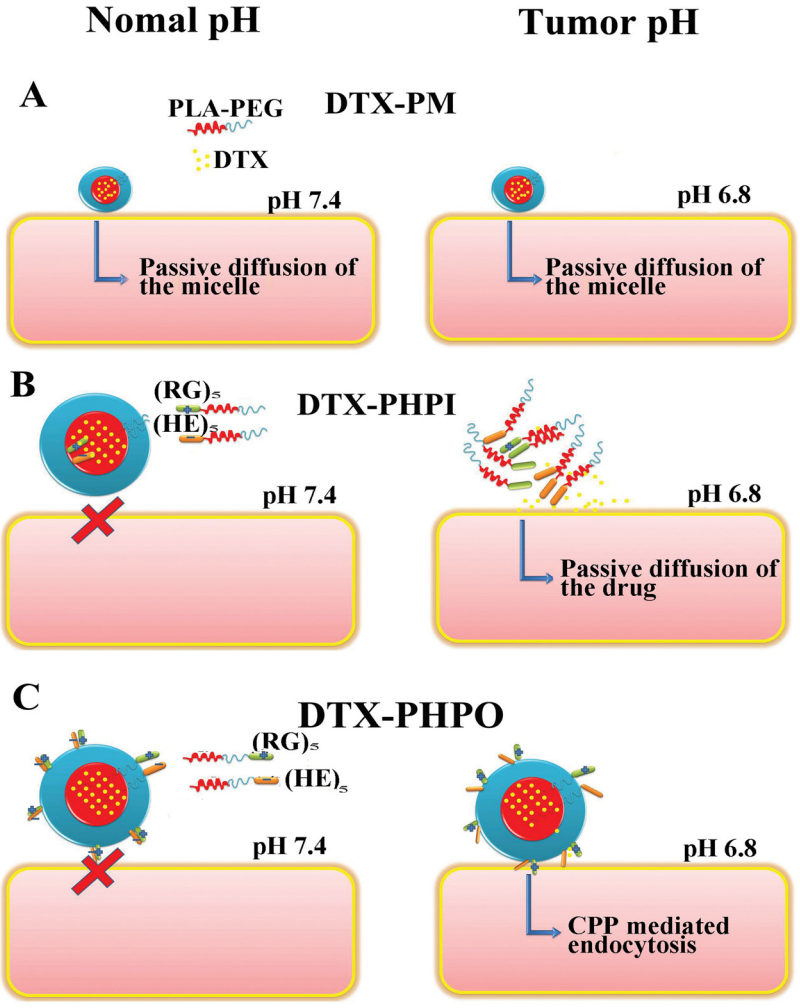

Fig. 1. Schematic representation showing the application of the different prepared polymeric micelles and their behaviors at the surface of both normal cells (pH 7.4) and tumor cells (pH 6.8): (A) DTX-PM, (B) DTX-PHPI, and (C) DTX-PHPO.