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A novel delivery system of doxorubicin with high load and pH-responsive release from the nanoparticles of poly (α,β-aspartic acid) derivative

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ABSTRACT

A poly (amino acid)-based amphiphilic copolymer was utilized to fabricate a better micellar drug delivery system (DDS) with improved compatibility and sustained release of doxorubicin (DOX). First, poly (ethylene glycol) monomethyl ether (mPEG) and DOX were conjugated onto polyasparihyazide (PAHy), prepared by hydrazinolysis of the poly (succinimide) (PSI), to afford an amphiphilic polymer [PEG-*hyd*-P (AHy-*hyd*-DOX)] with acid-liable hydrazone bonds. The DOX, chemically conjugated to the PAHy, was designed to supply hydrophobic segments. PEGs were also grafted to the polymer via hydrazone bonds to supply hydrophiphilic segments and prolong its lifetime in blood circulation. Free DOX molecules could be entrapped into the nanoparticles fabricated by such an amphiphilic polymer (PEG-*hyd*-P (AHy-*hyd*-DOX)), via hydrophobic interaction and π–π stacking between the conjugated and free DOX molecules to obtain a pH responsive drug delivery system with high DOX loaded. The drug loading capacity, drug release behavior, and morphology of the micelles were investigated. The biological activity of micelles was evaluated *in vitro*. The drug loading capacity was intensively augmented by adjusting the feed ratio, and the maximum loading capacity was as high as 38%. Besides, the DOX-loaded system exhibited pH-dependent drug release profiles *in vitro*. The cumulative release of DOX was much faster at pH 5.0 than that at pH 7.4. The DOX-loaded system kept highly antitumor activity for a long time, compared with free DOX. This easy-prepared DDS, with features of biocompatibility, biodegradability, high drug loading capacity and pH-responsiveness, was a promising controlled release delivery system for DOX.

1. Introduction

Many strategies have been proposed to fabricate nanocarriers for drug delivery, so-called drug delivery systems (DDSs) (Ali et al., 2011; Tong and Cheng, 2007), such as polymer prodrugs (Bildstein et al., 2011; Stella and Nti-Addae, 2007; Storm et al., 2008), dendrimers (Wijagkanalan et al., 2011), nanogels (Hamidi et al., 2008), liposomes and nanoparticles (Boudier et al., 2009; Mora-Huertas et al., 2010; Mudshinge et al., 2011; Napier and Desimone, 2007). Among all these, polymeric nanoparticles represent the most promising strategy for delivering water-insoluble agents for their therapeutic advantages including enhancing drug accumulation at the tumor tissues via the enhanced permeability and retention (EPR) effect, prolonging blood circulation time and fewer side effects (Mudshinge et al., 2011).

Typically, polymeric nanoparticles (Mikhail and Allen, 2009) are characterized by self-assembled from amphiphilic copolymers to form a core–shell structure with a hydrophobic inner core for drug entrapment and a hydrophilic out shell for stability (Jones and Leroux, 1999). Since drugs are considered to be incorporated into the inner core via the hydrophobic interaction (Bae and Kataoka, 2009), the compatibility between the drugs and core-forming segments may be a key factor for drug loading capacity. However, for most nanoparticles, the hydrophobic polymeric components are inert and have no other direct therapeutic efficacy, the distinct physicochemical properties of polymer blocks and their cargo lead that the loading contents are generally no more than 10% (Shen et al., 2010). This low drug loading content in many cases limits their clinical applications (Storm et al., 2008). For instance, the needed dose with a large amount of nanocarriers may increase the formulation viscosity, cause unexpected side effects (Li et al., 2011) such as toxicity and inflammation, and repeated administrations can lead to an extra burden for the patients (MacKay et al., 2009).

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Therefore, great efforts have been made to circumvent these problems. For example, various hydrophobic drug molecules, such as camptothecin (Shen et al., 2010), docetaxel (Li et al., 2011), and doxorubicin (DOX) (Yoo and Park, 2004), were directly used as the hydrophobic part of the block copolymer to minimize the use of other inert materials and thus increased the drug loading capacity. Researchers (Fukushima et al., 1999; Lee et al., 2007; Mikhail and Allen, 2010; Yokoyama et al., 1998; Yoo and Park, 2004) also employed chemical modification to modulate the drug-polymer compatibility. For instance, a series of research (Lammers et al., 2011; Nakanishi et al., 2001; Yokoyama et al., 1998) by Kataoka demonstrated that coupling anticancer drug DOX molecules to the side chains would highly increase drug-loading capacity, due to the intermolecular interactions between covalently attached drug and physically entrapped drugs. Similarly, Mikhail and Allen (2010) and Lee et al. (2007) tried to chemically conjugate hydrophobic drug molecules to the end of the hydrophobic block to improve drug-polymer compatibility. Their results had shown that coupled drug molecules could improve drug compatibility, and thus increase drug-loading capacity. However, studies have demonstrated that drug molecules conjugated via noncleavable linkages would loss their biological activity and affect the release of the drugs at the tumor (Fukushima et al., 1999; Yokoyama et al., 1998). Hydrazone linkage (Bae and Kataoka, 2009) is fairly stable at neutral pH but rapidly hydrolyzes in the acidic environment at pH < 6.8, which can be formed under very mild conditions. Recently, hydrazone-based coupling method has been widely used to develop pH-triggered controlled release system, since cancer tissues or cells have a slightly acidic pH (Bae et al., 2003; Bae et al., 2005; He et al., 2010; Hruby et al., 2005; Lee et al., 2008; Zhou et al., 2011). Nevertheless, there is rarely pH-sensitive controlled release system utilizing highly water-insoluble drugs themselves as the hydrophobic region to embed more drugs. This design can avoid the use of other hydrophobic components, maintain the covalently attached drug bioactivity and realize controlled release of anticancer drugs.

In this paper, we reported self-assembled DOX-loaded nanoparticles of a drug-conjugate copolymer (PEG-*hyd*-P (AHy-*hyd*-DOX)) for pH-triggered DOX release. The DOX, chemically conjugated to the water-soluble polymer of the PAHy via hydrazone bonds, was designed to supply hydrophobic segments and improve the compatibility between the core and physically entrapped DOX, and substantially increase DOX loading capacity. At the same time, to avoid hindering the cellular uptake, PEG was also grafted to the polymer by hydrazone linkage to form a removable shield (Storm et al., 2008).

Besides, for delivery systems, biodegradability should be required for the polymeric matrix in vivo applications. A series of hydrazine-bearing copolymers, especially the poly (amino acids), for drug conjugating, have gained a great interest and applied for the delivery of drugs. However, the synthetic processes often involved complicated preparation of monomers or other complex steps of protecting the side groups of polymers. In this aim, Polyasparthydrazide (PAHy) (Mendichi et al., 1999; Paolino et al., 2008), a derivative of poly (α,β-aspartic acid) (PAsp), prepared by hydrazinolysis of the PSI, was chosen for fabricating the micelles due to its highly water solubility, biodegradable, nontoxic, nonantigenic, easily produced in large quantities at reasonable cost, and the hydrazine side groups for formation of hydrazone bonds. Hence, using PAHy for conjugation has obvious advantages over many other reported polypeptides, which need complicated synthesis of monomers. What's more, the hydrolysis of the PEG shell and chemical conjugated DOX from the backbone of this water-soluble polymer, triggered by change in environment pH, would disrupt the nanocarriers and thus promote the diffusivity of the physical DOX from the hydrophobic core.

2. Materials and methods

2.1. Materials

Poly (ethylene glycol) monomethyl ether (CH_3O–PEG–OH, M_n = 5000) was purchased from Sigma–Aldrich. DOX hydrochloride was obtained from Beijing Huafeng United Technology CO., Ltd. and was used as received. Hydrazine hydrate aqueous solution (80%) was purchased from Tianjin Chemical Reagent. poly (succinimide) (PSI) (PDI = 1.3, M_n = 2.1 kD) was synthesized in our laboratory, according to the reference Kang et al. (2001). Other chemicals were of analytical reagent grade.

2.2. Synthesis of polyasparihyazide (PAHy)

PAHy was synthesized from PSI by diamid hydrate according to the previous report with little modification (Mendichi et al., 1999). Briefly, 10.23 g of hydrazine hydrate aqueous solution in 5 ml of DMF was added drop-wise to a continuously stirred solution of PSI (0.5 g, 3 mmol) in 8 ml of DMF. The reaction temperature was maintained at 22–26 °C for 4 h. The mixture was precipitated with excess diethyl ether and washed with acetone. The rough product was then dissolved in DI water, transferred to a dialysis membrane (MWCO 12 k–14 k Da) and purified by exhaustive dialysis against distilled water. The purified product was freeze-dried and kept at 4 °C (yield: 80%).

2.3. Synthesis of aldehyde–PEG (mPEG–CHO)

mPEG–CHO was prepared by oxidation of mPEG with DMSO/acetic anhydride referring to the literature (Cheng et al., 2009). mPEG (6 g) was dissolved in 20 ml of anhydrous DMSO and 1 ml of $CHCl_3$, followed by addition of 4 ml of acetic anhydride. The resultant mixture was stirred for 12 h at room temperature and then precipitated with excess diethyl ether. After drying under vacuum, white powder of mPEG–CHO was obtained (yield: 83%). ^{1}H NMR ($CDCl_3$): δ 9.75 (s, –CHO), 3.82 (t, $-CH_2-$CHO), 3.67 (m, PEG main chain), 3.38 (s, CH_3O–).

2.4. Synthesis of mPEG-hydrazone-PAHy (mPEG-hyd-PAHy)

PAHy (200 mg, 1.55 mmol of AHy repeat) was dissolved in 8 ml DI water. The pH value of reaction mixture was adjusted to about 5 with 0.1 N HCl, followed by adding of mPEG–CHO (408 mg, 0.081 mmol) to the solution. The reaction was then allowed to stand for 20 h at room temperature. mPEG-*hyd*-PAHy copolymer was obtained by dialysis against distilled water using a dialysis membrane (MWCO 12 k∼14 k Da).

2.5. Conjugation of DOX to mPEG-hyd-PAHy and preparation of DOX-loaded nanoparticles

Varying amount of DOX·HCl was dissolved in DMSO (23 ml) with mPEG-*hyd*-PAHy copolymer (30 mg). The mixed solution was stirred at room temperature for 24 h while being protected from light, followed by adding 2 equivalents of triethylamine (TEA). This mixture was stirred over night, and then extensively dialyzed against PBS (pH 7.4 10 mM) at room temperature for 1–2 days. The DOX-loaded nanoparticles in the dialysis tube was subsequently lyophilized and kept at 4 °C. Experiment without adding of TEA was taken as contrast to prepare PEG-*hyd*-P (AHy-*hyd*-DOX) nanoparticles (DOX-conjugated nanoparticles), which conjugated DOX by hydrazone bond without free DOX loaded (Bae et al., 2003).