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A novel targeting drug carrier to deliver chemical bonded and physical entrapped anti-tumor drugs

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ABSTRACT

In this study, we demonstrated a novel targeting drug carrier formed by amphiphilic prodrug based mixed micelle. Along with octadecyl chains, chemical bonded Dox moieties were utilized to entrap free drugs (PTX) and simultaneously acted as therapeutic agents. The formulation of CP-Folate/CPN = Dox/PTX showed spherical micellar structure and possessed high drug loading content, which was up to 22.9%. We examined the cell uptaken capacity and the cytotoxicity of mixed micelle by CLSM and MTT assay. The introducing of folate moiety enhanced intracellular accumulation in HeLa cells and co-delivery of Dox and PTX showed stronger anti-tumor activity even compared with free drugs.

1. Introduction

In the past decades, the basic research on chemotherapy has focused on the designing of all kinds of drug carriers (Batrakova and Kabanov, 2008; Mundargi et al., 2008; Slowing et al., 2008). The application of drug carriers can improve the water solubility of hydrophobic drugs, increase the intracellular drug accumulation and optimize the behavior of drug release (Allen et al., 1999; Kataoka et al., 2001; Moghimi et al., 2001). Conventional drug carriers are utilized to entrap single therapeutic agent, but in practical applications, combination delivery of multi-drugs or any other therapeutic agents is required to deal with complicated cases. For example, cocktails of drugs are also used to treat with HIV infection (Donati et al., 2004), and paclitaxel (PTX) and doxorubicin (DOX) can increase the inhibition of tumor growth (Gehl et al., 1996; Gustafson et al., 2005; Moghimi et al., 2001). Furthermore, combination delivery may hit different targets simultaneously, resulting enhanced therapy efficacy (Harries and Gore, 2002; Moghimi et al., 2001; Reich et al., 1999).

Successful combination delivery of multiply therapeutic agents is not only required a reasonable ratio of each component but also with adequate drug content. Therefore, the first problem in designing multi-drug carriers is to improve the drug loading content. Certain vehicles always involve a considerable amount of inert materials, which show no further functions except as the carrier matrices (Khandare and Minko, 2006). Thus, the percentage of drugs in the carriers is passively decreased. Recently, Shen's group prepared a prodrug based lipsome to encapsulate hydrophilic free drugs and this carrier showed a very high drug loading capacity which was up to 58% (Shen et al., 2010). Li and co-worker have synthesized a targeting prodrug nanoassembly, Biotin-PEG-Dox, to entrap hydrophobic free drugs (Yuan et al., 2013). These works provide us an idea to prepare high drug loading carriers, which is using the drugs as the materials of carrier.

Based on such ways, we present here a novel targeting drug platform to deliver multiply drugs (Scheme 1). Octadecyl-polyethylene glycol$_{100}$-hydrazone-doxorubicin (C_{18}-PEG-hy-Dox) was an amphiphilic prodrug to build up the main structure. A small amount of octadecyl-polyethylene glycol$_{100}$-Folate (C_{18}-PEG-Folate) was added as the targeting component. The moieties, octadecyl chain and Dox, could blend in aqueous solution by the hydrophobic effect to form mixed micelles, which were stabilized by the hydrophilic PEG blocks. Dox processed nearly half percentage of hydrophobic moieties and acted two roles here: the therapeutic agent and the carrier matrix. By utilizing such property, another model hydrophobic drug, PTX was loaded into micelle core. An acid-labile structure was introduced into the system to endow the carrier with pH-sensitive drug release behavior.

2. Experimental part

2.1. Materials

1,1′-Carbonyldiimidazole (CDI), dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) were purchased from

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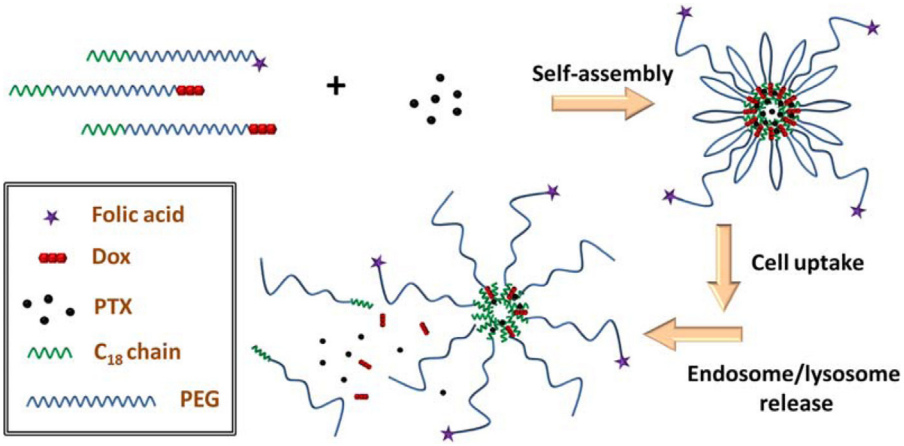

Scheme 1. The schematic illustration of structure of CP-Folate/CPN = Dox/PTX mixed micelle and the process of the targeted drug delivery.

Sinopharm (China) and used as received. Doxorubicin hydrochloride (Dox·HCl) and Brij S 100 (average Mn ~4670) were purchased from Sigma–Aldrich and used as received. Dimethyl sulfoxide (DMSO) was dried over 4 Å molecular sieve and distilled under vacuum. Dichloromethane (DCM) was distilled over CaH_2 before use. Methanol was dried over 3 Å molecular sieve and distilled by rectification. Bovine serum albumin (BSA), Dubelcco's Modified Eagle's Medium (DMEM), penicillin–streptomycin, trypsin, and phosphate-buffered saline (PBS) were purchased from GIBCO Invitrogen Corporation. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Hochest 33258 were purchased from Sigma–Aldrich.

2.1.1. C_{18}-PEG-CDI

Brij S 100 (4.67 g, 1.0 mmol) and CDI (1.62 g, 10.0 mmol) were dissolved in DCM (50 mL), and then the solution was allowed at room temperature for 24 h. The most portion of solvent was evaporated and the residue was poured into excess anhydrous diethyl ether. The white precipitate was collected by centrifugation, washed with diethyl ether and dried under vacuum. Yield 4.2 g, (89.9%). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.14 (s, 1H), 7.47 (d, 1H), 7.09 (d, 1H), 4.56 (t, 2H), 3.42–3.88 (m, 400H), 1.57 (m, 2H), 1.25 (m, 30H), 0.88 (t, 3H).

2.1.2. C_{18}-PEG-NH-NH_2

C_{18}-PEG-CDI (476 mg, 0.1 mmol), hydrazine (16 mg, 0.5 mmol) and triethylamine (101 mg, 1 mmol) were dissolved in 10 mL of DCM. The mixture was concentrated by rotary evaporation and the residue was poured into excess anhydrous diethyl ether to form precipitate. The white precipitate was collected by centrifugation, then washed with diethyl ether and dried under vacuum. Yield: 412 mg, (86.6%). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm), 4.27 (m, 2H), 3.42–3.88 (m, 400H), 1.57 (m, 2H), 1.25 (m, 30H), 0.88 (t, 3H).

2.1.3. Folate-NH_2

The synthesis of Folate-NH_2 was according to a published procedure (Lee and Low, 1995). Folic acid (441 mg, 1 mmol) was dissolved in 20 mL of DMSO, and then treated with DCC (248 mg, 1.2 mmol) and NHS (230 mg, 2.0 mmol). The mixture was stirring at 50 °C for 6 h. The resulting Folate-NHS was reacted with ethylenediamine (781 mg, 13.0 mmol) and pyridine (500 mg, 6.3 mmol) at room temperature overnight. The mixture was poured into excess acetonitrile, and the precipitate was collected and washed with diethyl ether before drying under vacuum to get yellow powder. Yield: 297 mg, (61.5%). The obtained Folate-NH_2 was directly used in the next step without further purification.

2.1.4. C_{18}-PEG-Folate (CP-Folate)

C_{18}-PEG-CDI (476 mg, 0.1 mmol), Folate-NH_2 (241 mg, 0.5 mmol) and triethylamine (0.21 g, 2.1 mmol) were dissolved in 10 mL of DMSO. The mixture was stirring at room temperature for 48 h, and then dialyzed extensively against with DI water for 72 h (Mw cutoff: 3500 Da). The CP-Folate was obtained by freeze-drying. Yield: 327 mg (63.5%).

2.1.5. C_{18}-PEG-NH-N = Dox (CPN = Dox)

Dox·HCl (87 mg, 0.15 mmol) and C_{18}-PEG-NH-NH_2 (476 mg, 0.1 mmol) were dissolved in 15 mL of anhydrous menthol, and treated with a drop of TFA. The solution was refluxed under dark for 48 and then cooled down to room temperature. The solvent was evaporated under vacuum and the residue was resolved in 10 mL of anhydrous DMSO. The solution was dialyzed against with DMSO for 48 h (Mw cutoff: 3500 Da) and then dialyzed extensively against with DI water to remove the organic solvent and any other impurities. The CPN = Dox was obtained by freeze-drying as dark red solid. Yield: 299 mg (57.2%).

2.2. Methods

^{1}H NMR spectra were recorded at 300 MHz on a Mercury VX-300 spectrometer by using tetramethylsilane (TMS) as the internal reference. A drop of micelle solution was placed onto a copper grid with carbon film and then stained by phosphotungstic acid. The TEM images were taken by JEM-2100 (HR) transmission electron microscope at an acceleration voltage of 200 keV. Size and distribution of the mixed micelles were measured by Dynamic Light Satter (Zetasizer, Malvern).

2.2.1. Determination of the content of Dox in CPN = Dox

The content of Dox in CPN = Dox was determined using fluorescence spectroscopy. The CPN = Dox was resolved in 1N HCl solution and kept in dark over night at room temperature. The content of Dox was calculated based on the fluorescence intensity at emission wavelength of 560 nm, excitation wavelength of 488 nm, and slit width of 5 nm calibrated by a standard curve of Dox·HCl.

2.2.2. Micelle preparation and drug encapsulation

The mixed micelles (CP-Folate/CPN = Dox or CP-OH/CPN = Dox) were prepared by dialysis. Typically, 1.0 mg of CPN = Dox and 0.2 mg of CP-Folate were stirring in 200 μL of DMSO, and then 1.8 mL of DI water was added dropwise into the above solution. The mixture was further stirred for 2 h and then dialyzed (Mw 3500, cutoff) against with DI water.