Journal of Controlled Release 230 (2016) 34–44

Contents lists available at ScienceDirect

Journal of Controlled Release

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

A small molecule nanodrug consisting of amphiphilic targeting ligand–chemotherapy drug conjugate for targeted cancer therapy

Quanbing Mou [1], Yuan Ma [1], Xinyuan Zhu *, Deyue Yan *

School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 1 March 2016
Accepted 28 March 2016
Available online 31 March 2016

Keywords:
Cancer therapy
Targeted delivery
Small molecule nanodrugs
Amphiphilic self-assembly

ABSTRACT

Targeted drug delivery is a broadly applicable approach for cancer therapy. However, the nanocarrier-based targeted delivery system suffers from batch-to-batch variation, quality concerns and carrier-related toxicity issues. Thus, to develop a carrier-free targeted delivery system with nanoscale characteristics is very attractive. Here, a novel targeting small molecule nanodrug self-delivery system consisting of targeting ligand and chemotherapy drug was constructed, which combined the advantages of small molecules and nano-assemblies together and showed excellent targeting ability and long blood circulation time with well-defined structure, high drug loading ratio and on-demand drug release behavior. As a proof-of-concept, lactose (Lac) and doxorubicin (DOX) were chosen as the targeting ligand and chemotherapy drug, respectively. Lac and DOX were conjugated through a pH-responsive hydrazone group. For its intrinsic amphiphilic property, Lac-DOX conjugate could self-assemble into nanoparticles in water. Both *in vitro* and *in vivo* assays indicated that Lac-DOX nanoparticles exhibited enhanced anticancer activity and weak side effects. This novel active targeting nanodrug delivery system shows great potential in cancer therapy.

1. Introduction

Targeted cancer therapy has expanded tremendously in recent years. Owing to the rapid development of nanotechnology, various active targeting nanocarriers based on the modification of nanoparticles with tumor-specific molecules have been carried out. These active targeting ligands including antibodies, peptides and small molecules (*e.g.*, folate [1], galactose [2], lactose [3]) can greatly enhance the cellular uptake of nanoparticles *via* receptor-mediated endocytosis. Nanocarriers used for cancer therapies such as liposomes [4–6], micelles [7–20], protein nanoparticles [21,22], metallic nanoparticles [23–27], inorganic nanoparticles [28,29] can passively accumulate in the tumor site through the enhanced permeability and retention (EPR) effect [30–34]. Both passive and active targeting properties endow the delivery systems with enhanced therapeutic activity. Nevertheless, the fabrication of active targeting nanocarriers is extremely complicated, including materials synthesis/assembly, ligand coupling, separation and purification, which could cause batch-to-batch variation and quality concerns [35]. Besides, the degradation, metabolism, and excretion of nanocarriers can cause significant toxicity issues [36] (*e.g.*, mitochondrial damage, cardiovascular effects, platelet aggregation, oxidative stress, inflammation). The active targeting nano drug delivery systems with high dose use of nanocarriers do increase the therapeutic efficiency, but nanocarriers used are potential hazards. Thus, there are only a few targeted nanodrug delivery systems in clinical trials [37]. It is an urgent demand to develop a carrier-free system with excellent targeting ability and weak side effects.

Different from the carrier-based targeted delivery systems, a carrier-free amphiphilic drug–drug conjugate (ADDC) self-delivery system from direct conjugation of hydrophobic anticancer drug with hydrophilic anticancer drug has been successfully developed in our group [38]. Inspired by the simple concept only using the amphiphilic small molecules to form micelles, here we develop an active targeting small molecule nanodrug consisting of the hydrophilic targeting ligand and the hydrophobic chemotherapy drug through a bio-responsive linkage. This novel active targeting self-delivery system brings together the antigen-targeting specificity of the targeting ligand, favorable pharmacokinetics of nanomedicine and the cytotoxic potency of promising chemotherapeutic drugs. Chemotherapeutic drugs can be released in stimuli circumstance. The drug loading ratio of this active targeting self-delivery system conjugates is high, for the molecular weights of targeting ligands and anticancer drugs are in the same order of magnitude. Besides, the toxic anticancer drug moieties of the conjugates are aggregated in the core of the micelles, and surrounded by the nontoxic targeting ligands, which leads to significant reduction of the cytotoxicity to normal cells. Moreover, the active targeting self-delivery system can accumulate at the tumor site both passively and actively.

As a proof-of-concept, lactose (a small molecular weight hydrophilic hepatocyte targeting molecule, Lac) [39–41] and doxorubicin (a hydrophobic anticancer drug, DOX) are simply conjugated *via* a pH-responsive hydrazone group [42–45] (Lac-DOX) to form an

* Corresponding authors.
E-mail addresses: xyzhu@sjtu.edu.cn (X. Zhu), dyyan@sjtu.edu.cn (D. Yan).
[1] Both authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.jconrel.2016.03.037
0168-3659/

active targeting nanodrug delivery system, as illustrated in Scheme 1. Similar to ADDC, Lac-DOX conjugate is expected to show favorable pharmacokinetics and benefits of highly potent anticancer drugs. The structure of Lac-DOX conjugate is well-defined with drug loading ratio of 61.7%. Lac-DOX conjugate self-assembles into nanoparticles for its intrinsic amphiphilic property. Self-assembled Lac-DOX nanoparticles can passively accumulate in the tumor site through EPR effect. More importantly, Lac can greatly enhance the cellular uptake of nanoparticles *via* receptor-mediated endocytosis. Both passive and active targeting properties endow the delivery system with enhanced therapeutic activity.

2. Materials and methods

2.1. Materials

Dimethylsulfoxide (DMSO) was dried over calcium hydride for 48 h and then distilled before use. Methanol was purchased from Hipure Chem and was high performance liquid chromatography (HPLC) grade. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), 4-O-beta-D-galactopyranosyl-D-gluconic acid (Lactobionic acid, 97%, Aladdin), hydrazine hydrate (98%, Aladdin), and delta-gluconolactone (99%, Adamas) were used as received. Doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Huafeng United Technology Corporation.

2.2. Measurements

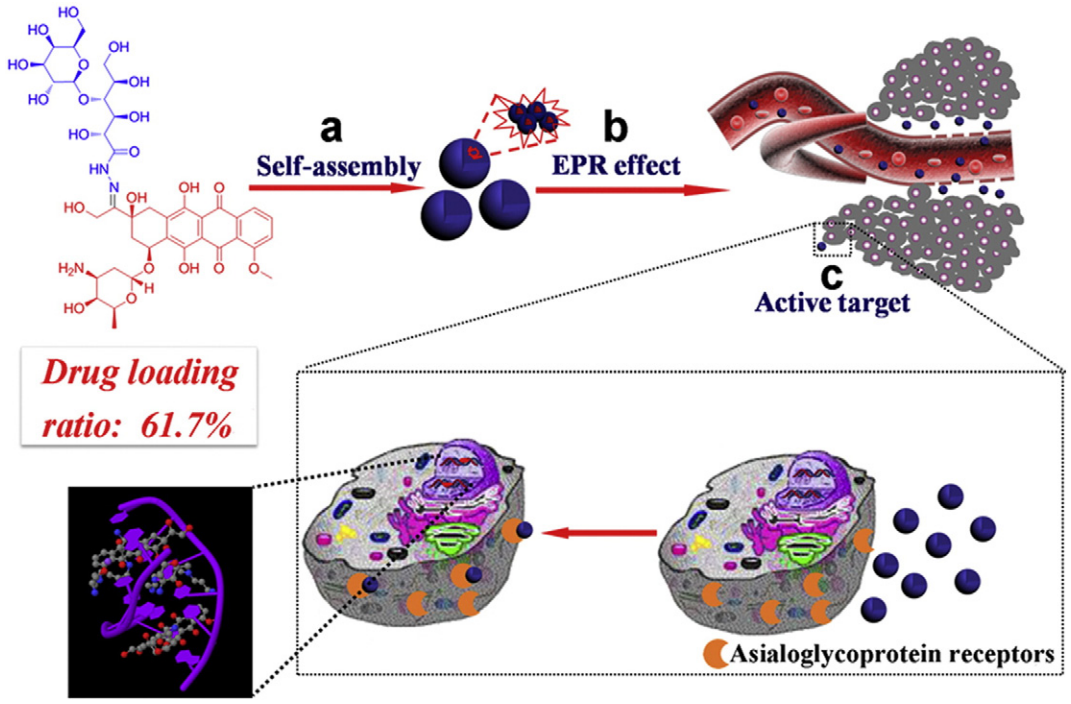
Nuclear magnetic resonance (NMR) spectroscopy spectra were performed on a Varian Mercury Plus 400 MHz spectrometer with deuterium oxide (D_2O) or dimethylsulfoxide-d6 (DMSO-d_6) as solvents. Ultraviolet–visible absorption (UV–vis) spectra were collected on an Evolution 300 UV–vis spectrophotometer. Fourier transform infrared (FT-IR) spectra were measured on a Paragon 1000 instrument. High-resolution mass spectra (HRMS) were recorded on a Waters-ACQUITYTM UPLC & Q-TOF-MS premier. The fluorescent spectra of sample solutions were performed on a Perkin-Elmer LS-50B fluorescence spectrometer. The excitation wavelength was set at 488 nm, which was chosen according to the maximum intensity obtained in the excitation spectra. Step increment was set as 2 nm, and scan speed was set at 480 nm/min. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer NanoS apparatus (Malvern Instruments, Ltd.) equipped with a 4 mW laser light operating at $\lambda = 633$ nm. All samples were measured at a scattering angle of 90°. The morphology and size of nanoparticles were characterized by a Tecnai G2 Spirit Biotwin instrument at voltage of 120 kV.

2.3. Synthesis of lactobionolactone

According to the literature procedure [46] with a little modification, lactobionic acid (5.0000 g, 13.96 mmol) was dissolved in anhydrous methanol (70.0 mL) at 75 °C followed by vacuum distillation at 40 °C. The procedure was repeated until lactobionic acid was fully converted to lactobionolactone. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 5.33–4.01 (br, OH), 4.34–4.13 (m, 2H, CH), 4.02–3.86 (m, 1H, CH), 3.75–3.25 (m, 10H, CH & CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.44, 105.42, 84.19, 76.01, 73.66, 71.83, 71.63, 71.41, 70.94, 68.55, 62.64, 60.79. HRMS: (ESI) $[M-H]^-$ calcd. for $C_{12}H_{19}O_{11}$, 339.0927, found 339.0927.

2.4. Synthesis of (2R,3R,4R,5R)-2,3,5,6-tetrahydroxy-4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)hexanehydrazide (Lac-NHNH$_2$)

Lactobionolactone (3.0000 g, 8.82 mmol) was dissolved in anhydrous methanol (40.0 mL) at 25 °C. Hydrazine hydrate (2.2270 g, 44.10 mmol) was added dropwise to the solution of lactobionolactone *via* a syringe and keep at 25 °C for 1 h. The white powder was collected by a rotary evaporator (yield: 76.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.91–8.50 (s, 1H, NH), 5.23–4.03 (br, OH), 4.32–4.17 (d, J = 4.27 Hz, 1H, CH), 4.19–4.09 (d, J = 4.16 Hz, 1H, CH), 4.04–3.93 (m, 1H, CH), 3.73–3.60 (m, 2H, CH), 3.60–3.46 (m, 5H, CH & CH_2), 3.45–



Scheme 1. Lac-DOX nanoparticles for passive and active targeting drug delivery. (a) Schematic illustration for the self-assembly of amphiphilic Lac-DOX. (b) Lac-DOX nanoparticles accumulated at the tumor site through both passive and active targeting mechanisms. (c) Free DOX was released from Lac-DOX nanoparticles to inhibit the proliferation of SMMC-7721 cells.