Journal of Controlled Release 261 (2017) 113–125

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Redox-sensitive and hyaluronic acid functionalized liposomes for cytoplasmic drug delivery to osteosarcoma in animal models

Yingying Chi[a], Xuelei Yin[a], Kaoxiang Sun[a], Shuaishuai Feng[a], Jinhu Liu[a], Daquan Chen[a], Chuanyou Guo[b,⁎⁎], Zimei Wu[a,c,⁎]

[a] School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, PR China
[b] Department of Bone Joint, Qingdao Municipal Hospital, Qingdao, Shandong Province 266011, PR China
[c] School of Pharmacy, University of Auckland, Auckland 1142, New Zealand

A R T I C L E I N F O

Keywords:
Hyaluronic acid (HA)
CD44
GSH-responsive
PEG-detachment
Intracellular delivery
Long-circulation
Osteosarcoma

A B S T R A C T

This study aimed to develop redox-sensitive and CD44-targeted liposomes to improve chemotherapy of osteosarcoma. Cationic liposomes were prepared and stabilized with a novel detachable polyethylene glycol (PEG_{2000}) conjugated with cholesterol through a bio-reducible disulfide linker (Chol-SS-mPEG). Hyaluronic acid (HA, MW 20–40 kDa), a ligand to CD44, was non-covalently coated on the cationic liposomes. Doxorubicin (DOX) was actively loaded in the liposomes as a model drug. The roles of HA and Chol-SS-mPEG on intracellular drug delivery efficiency, and antitumor efficacy were studied. The structure of Chol-SS-mPEG was confirmed with Fourier-transform infrared and nuclear magnetic resonance (^{1}H NMR). The liposomes, Chol-SS-mPEG/HA-L had a mean diameter of 165 nm, zeta potential − 28.9 mV, and destabilized in reducing or acidic (pH 5–6) conditions. *In vitro* release of DOX was well-controlled at physiological conditions, but a burst release of 60% was observed in the presence of 10 mM glutathione (GSH), in contrast to non-redox sensitive liposomes (Chol-mPEG/HA-L and Chol-mPEG-L). MTT cell viability assay showed that the dual-functional Chol-SS-mPEG/HA-L with a drug loading of 15.0% (w/w) had significantly higher cytotoxicity to MG63 osteosarcoma cells compared with non-reduction sensitive or non-HA coated liposomes ($p < 0.01$), consistent with the cellular uptake and intracellular trafficking studies using confocal microscopy and flow cytometry. Furthermore, the HA-coated GSH-responsive liposomes preferentially internalized to MG63 over human liver cells LO2. In rats, liposomes stabilized with either Chol-SS-mPEG or Chol-mPEG, with or without HA, increased the half-life of DOX by > 10-fold. In a MG63 xenograft mouse model, Chol-SS-mPEG/HA-L showed the most effective tumor suppression with minimal uptake by the liver compared with other liposomes. All animals treated with liposomal formulations survived, in contrast to those free-DOX treated. In conclusion, the easily prepared Chol-SS-mPEG/HA-L was demonstrated as an excellent CD44-mediated intracellular delivery system capable of long-circulation and GSH-triggered cytoplasmic drug release. Further translational and multidisciplinary research is required to make it real clinical benefits to cancer patients.

1. Introduction

Nano-sized liposomes have been extensively investigated for cancer drug delivery due to their abilities to protect drug from enzymatic degradation, and potentials to accumulate in solid tumors [1,2]. Compared with other nano-sized delivery systems, such as polymeric micelles and nanogels, liposomes have advantages including good biocompatibility, and the versatility of loading both hydrophilic and hydrophobic drugs with high payloads [3,4]. Furthermore, the surface of liposomes can be modified with ligands to augment their specific cellular uptake ("active tumor targeting") [5]. To prevent rapid clearance by the reticuloendothelial system (RES) thus prolonging *in vivo* circulation, liposomes are coated with hydrophilic polymers such as polyethylene glycol (PEG, *i.e.* PEGylation) [1,4]. To further improve the therapeutic outcomes by promoting drug release at the target, recent efforts have been made in developing stimuli-sensitive drug release systems, including sheddable PEG-coating by exploiting the microenvironmental stimuli in tumor such as redox potential and low pH [6,7].

⁎ Correspondence to: Zimei Wu, School of Pharmacy, University of Auckland, Auckland 1142, New Zealand.
⁎⁎ Corresponding author.
E-mail address: z.wu@auckland.ac.nz (Z. Wu).

http://dx.doi.org/10.1016/j.jconrel.2017.06.027
Received 4 April 2017; Received in revised form 25 June 2017; Accepted 26 June 2017
Available online 27 June 2017
0168-3659/ © 2017 Elsevier B.V. All rights reserved.

Nanoparticulate systems comprising polymers containing disulfide bonds (−SS−) have been employed for targeted drug release using the intracellular redox potential of the tumors as stimuli [8,9]. The disulfide bond can be cleaved by the tumoral intracellular glutathione (GSH, 2–10 mM), a strong biological reducing agent, while remaining stable in a predominantly oxidizing extracellular space, where GSH concentration is much lower, 2–20 μM [10]. Moreover, because of the frequently multiple genetic alterations of cancer cells, the GSH concentration in tumor was found to be > 4-fold higher than that in normal cells [11]. Therefore, owing to these discrepancies in GSH distribution disulfide bonds containing nanoparticles can be preferentially cleaved in cancer cells, releasing the payload intracellularly.

Hyaluronic acid (HA), an endogenous polysaccharide, is a well-identified principal ligand for cluster of differentiation 44 (CD44). CD44 is a family of cell-surface glycoproteins involved in cell-cell interactions, cell adhesion, and migration in many malignant tumors [12,13]. Overexpression of CD44 on cancer cells enhances tumor aggressiveness by increasing adhesion to its extracellular matrix ligand HA [14]. CD44 also has low degree of expression in a number of normal cell types but these cells are not in direct contact with blood flow [15]. Therefore, HA has become an attractive ligand for intracellular delivery to CD44-overexpressing tumors by exploiting ligand-receptor interaction and has been proven to enhance antitumour efficacy in preclinical models [5,16,17]. Moreover, HA are hydrophilic polymers, HA-coating endows the nanocarriers such as liposomes with long-circulation *in vivo*, although not as significant as PEGlyation [18].

Osteosarcoma (OS) is the most common primary malignant bone tumor that usually affects children and adolescents. The treatment for OS includes chemotherapy and surgery [19,20]. The large-scale surgery (amputation) did not result in drastic improvements [19]. Chemotherapy at high doses is essential for long-term survival of OS patients, usually with multiple chemotherapeutic agents. However this result in severe side effects in patients due to the indiscriminate drug distribution in the body and low specificity [20]. Therefore, tumor targeted drug delivery demonstrated may be a promising approach to improve OS treatment in animal models [21].

In this work, a novel sheddable PEG conjugated with cholesterol through a disulfide (−SS−) bond, Chol-SS-mPEG, was designed and synthesized. The bio-reducible polymer was used to develop a redox-sensitive liposomal system, to which HA was further coated as hydrophilic shell as well as a CD44 ligand on the surface of liposomes. CD44 is an important target of OS [12,13,22]. The doxorubicin (DOX)-loaded Chol-SS-mPEG/HA-L were envisaged to have long circulation and accumulate in OS, followed by internalization into CD44 over-expressing OS cells *via* CD44-mediated endocytosis; once in the cells, DOX would be released in response to the intracellular GSH as a result of PEG-detachment (Scheme 1). The roles of HA and redox-sensitivity on OS specific intracellular drug delivery efficiency were systemically characterized *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Monomethoxy PEG-amine ($mPEG_{2000}$-NH_2) was purchased from Yare Biotech, Co., Ltd. (Shanghai, China). Hyaluronic acid (HA; molecular weight 20–40 kDa) was purchased from Shandong Biopharmaceutical Co., Ltd. (Shandong, China). Doxorubicin hydrochloride (DOX), cholesterol, *N*-hydroxysuccinimide (NHS), D,L-dithiothreitol (DTT), 3,3′-dithiodipropionic acid (DTOP), 4-dimethylaminopyridine (DMAP), soybean phosphatidylcholine (SPC), and dialysis bags (MWCO 2000 Da or 10 kDa) were purchased from Aladdin (Shanghai, China). Lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-3-trimethylammonium-propan (DOTAP) were purchased from Avanti Polar Lipids (Alabaster, AL, USA) while 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was from Dojindo Laboratories (Tokyo, Japan). Methanoic (formic) acid, dimethyl sulfoxide (DMSO), *N*, *N*-dimethylformamide (DMF) and other chemicals were of analytical reagent grade.

For cell culture studies, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Hoechst 33342 were obtained from Sigma-Aldrich (USA). Fetal bovine serum (FBS), RPMI-1640 medium, McCoy's 5A medium were purchased from Hyclone (Utah, USA) and used as received. Mouse osteosarcoma MG63 cells were purchased from BeNa Culture Collection (Beijing, China). Normal human hepatic LO2 cell line was gift from Medicine & Pharmacy Research Center, Binzhou Medical University.

For animal studies, Sprague-Dawley (SD) male rats of 7–9 weeks old weighing 190–210 g and male BALB/c nude mice (18–20 g) were from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The experiments were carried out in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All animal studies were approved by the Experimental Animals Administrative Committee of Yantai University.

2.2. Polymer synthesis

2.2.1. Synthesis of Chol-SS-mPEG

To prepare Chol-SS-mPEG, Chol-SS-COOH was first synthesized by conjugating cholesterol with DTOP through a DCC/DMAP coupling reaction (Scheme 2). First, DTOP (0.6 g, 3.0 mmol) in anhydrous DMF was activated with *N*, *N*′-dicyclohexyl carbodiimide and (DCC, 0.9 g, 4.5 mmol) and 4-dimethylaminopyridine (DMAP, 0.5 g, 4.5 mmol) under stirring for 6 h at room temperature, to form active esters of docosapentaenoic acid (DPA). Then 1.0 g of cholesterol in anhydrous DMF (30 mg/mL) was added dropwise and the solution was stirred at 70 °C for 48 h under N_2 atmosphere while the progress of reaction was monitored by thin-layer chromatography (TLC) with a mobile liquid solvent phase, $CH_3COCH_3/CHCl_3$ (1:1, v/v). The solution containing Chol-SS-COOH was dialyzed (MWCO 500 Da) against an excess amount of distilled water for 12 h to remove the water-soluble impurities. The resulting solution was lyophilized and re-dissolved in ethyl acetate and applied to the silica gel column. The column was washed with petroleum ether-ethyl acetate mixtures (1:10, v/v). The obtained product was dried under reduced pressure. Finally, the product was further confirmed with ^{1}H NMR spectra using a Bruker AVIII 500 NMR Spectrometer. Deuterated DMSO-*d6* was used as the solvent. The peaks for cholesteryl moiety, $-S-S-CH_2-$ and −COOH were of particular interest.

Chol-SS-COOH was used to synthesize Chol-SS-mPEG also using a DCC/DMAP coupling reaction by which an amide bond was formed between the carboxyl group of Chol-SS-COOH and the amine group of mPEG-NH_2 (Scheme 2A). Chol-SS-COOH (0.6 g, 1.0 mmol) was dissolved in 10 mL of DMF. Then the coupling agents DCC (0.3 g, 1.5 mmol) and DMAP (0.3 g, 1.5 mmol) were added and the solution was kept at room temperature under stirring for 4 h. Then 2.0 g of mPEG-NH_2 was added and dissolved. The solution was stirred at room temperature for 24 h. The solution was dialyzed against an excess amount of distilled water with a dialysis bag (MWCO 7000) for 12 h. The product was lyophilized to obtain the final produce Chol-SS-mPEG before subjected to ^{1}H NMR analysis. The yield of Chol-SS-mPEG was 60.5%.

2.2.2. Synthesis of Chol-mPEG

To obtain Chol-mPEG, first, Chol-COOH was synthesized according to the previously described method [23] with some modification (Scheme 2B). Briefly, cholesterol (3.86 g, 10 mmol) and succinic anhydride (1.00 g, 10 mmol) were dissolved in 20 mL of pyridine in a three-necked flask equipped with a reflux condenser. The reaction mixture was stirred magnetically in an oil bath at 65 °C for 3 days under reflux. Then 25 mL of acetone at 55 °C was added, followed by filtration to remove the insoluble matter. The filtrate was stored at − 20 °C

Download English Version:

https://daneshyari.com/en/article/5433427

Download Persian Version:

https://daneshyari.com/article/5433427

Daneshyari.com