Journal of Controlled Release 244 (2016) 229–239

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Bioresponsive and fluorescent hyaluronic acid-iodixanol nanogels for targeted X-ray computed tomography imaging and chemotherapy of breast tumors

Yaqin Zhu [a,b], Xiuxiu Wang [a], Jing Chen [a], Jian Zhang [a], Fenghua Meng [a], Chao Deng [a], Ru Cheng [a], Jan Feijen [a,b,*], Zhiyuan Zhong [a,*]

[a] Biomedical Polymers Laboratory, and Jiangsu Key Laboratory of Advanced FunctionalPolymer Design and Application, College of Chemistry, Chemical Engineering and MaterialsScience, Soochow University, Suzhou, 215123, PR China

[b] Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, P.O. Box 217, 7500, AE, Enschede, The Netherlands

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form 18 August 2016
Accepted 22 August 2016
Available online 25 August 2016

Keywords:
Nanogels
Hyaluronic acid
X-ray computed tomography
Reduction-sensitive
Cancer
Paclitaxel

ABSTRACT

Nanotheranostics is a rapidly growing field combining disease diagnosis and therapy, which ultimately may add in the development of 'personalized medicine'. Here, we designed and developed bioresponsive and fluorescent hyaluronic acid-iodixanol nanogels (HAI-NGs) for targeted X-ray computed tomography (CT) imaging and chemotherapy of MCF-7 human breast tumors. HAI-NGs were obtained with a small size of ca. 90 nm, bright green fluoresence and high serum stability from hyaluronic acid-cystamine-tetrazole and reductively degradable polyiodixanol-methacrylate via nanoprecipitation and a photo-click crosslinking reaction. Notably, paclitaxel (PTX)-loaded HAI-NGs showed a fast glutathione-responsive drug release. Confocal microscopy displayed efficient uptake of HAI-NGs by CD44 overexpressing MCF-7 cells via a receptor-mediated mechanism. MTT assays revealed that HAI-NGs were nontoxic to MCF-7 cells even at a high concentration of 1 mg/mL whereas PTX-loaded HAI-NGs exhibited strong inhibition of MCF-7 cells. The *in vivo* pharmcokinetics, near-infrared imaging and biodistribution studies revealed that HAI-NGs significantly prolonged the blood circulation time and enhanced tumor accumulation of PTX. Interestingly, significantly enhanced CT imaging was observed for MCF-7 breast tumors in nude mice via either intratumoral or intravenous injection of HAI-NGs as compared to iodixanol. HAI-NGs fluoresence was distributed thoughout the whole tumor indicating deep tumor penetration. PTX-loaded HAI-NGs showed effective suppression of tumor growth with little systemic toxicity. HAI-NGs appear as a "smart" theranostic nanoplatform for the treatment of CD44 positive tumors.

1. Introduction

Theranostic nanomedicines contain both a diagnostic agent and one or more therapeutic drugs within one integrated system, enabling non-invasive diagnosis, therapy, and real-time monitoring of the therapeutic response at the same time [1–4]. Among various imaging techniques, computed tomography (CT) is one of the most commonly used non-invasive clinical imaging modalities because of its wide availability, high spatial resolution, unlimited depth, and accurate anatomical information with reconstructed three dimensional imaging [5–7]. Iodixanol (Visipaque) is a small iodinated molecule, clinically used as a CT contrast agent that has a low osmolality and great tolerability [8]. However, like all low molecular weight iodinated CT contrast agents, iodixanol has drawbacks like non-specific distribution and rapid renal clearance following *i.v.* injection [9]. In recent years, nanosized CT contrast agents have attracted great interest as they have several advantages over small molecular contrast agents such as prolonged circulation time, site-specific accumulation and use for theranostics [10–13]. Some recent work showed systems with great promise of nanosized CT contrast agents such as iodinated hyaluronic acid oligomer-based nano-assembled systems, theranostic self-assembly structures of gold nanoparticles, and multifunctional dendrimer-entrapped gold nanoparticles for simultaneous tumor imaging and therapy [14–16].

Among various types of nanoscale drug delivery systems, nanogels have attracted increasing attention since they have a large surface area for multivalent bioconjugation and a crosslinked three-dimensional network structure that offers great colloidal stability [17–19]. To

* Corresponding authors at: Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, 215123, PR China.

E-mail addresses: j.feijen@tnw.utwente.nl (J. Feijen), zyzhong@suda.edu.cn (Z. Zhong).

http://dx.doi.org/10.1016/j.jconrel.2016.08.027
0168-3659/

achieve rapid release of the payload at the target site, pH, redox potential, and enzyme-responsive nanogels have been designed [20–27]. Nanogels based on hyaluronic acid (HA) have recently appeared as a unique system because HA is a hydrophilic natural material with excellent biocompatibility and intrinsic targeting ability toward CD44-overexpressing tumor cells [26,28–31]. HA nanoparticles have been used for efficient delivery of chemotherapeutics, proteins as well as siRNA *in vitro* and *in vivo* [32–35].

In this paper, we report on bioresponsive and fluorescent hyaluronic acid-iodixanol nanogels (HAI-NGs) for targeted CT imaging and chemotherapy of MCF-7 human breast tumor (Scheme 1). HAI-NGs were obtained from hyaluronic acid-cystamine-tetrazole (HA-Cys-Tet) and reductively degradable polyiodixanol-methacrylate (SS-PI-MA) *via* nanoprecipitation and a photo-click crosslinking reaction. HAI-NGs were designed with the following unique features: i) both HA and iodixanol have excellent biocompatibility and are currently used in the clinic; ii) the "tetrazole-ene" photo-click crosslinking reaction is highly selective, which prevents cross-reaction with most drugs and furthermore endows nanogels with bright green fluorescence [36,37]; iii) HA can actively target CD44 receptors which are overexpressed on various malignant tumor cells and stem cells [38–41]; iv) HAI-NGs can be used for targeted CT imaging *in vivo*; and v) the reduction-sensitivity of HAI-NGs allows fast intracellular release of payloads like PTX to achieve efficient and targeted chemotherapy. Herein, the stability of HAI-NGs and the reduction-triggered PTX release from PTX loaded HAI-NGs were investigated. Furthermore, the targetability of HAI-NGs and antitumor activity of PTX loaded HAI-NGs toward MCF-7 cells, the pharmacokinetics and biodistribution, NIR and CT imaging, as well as therapeutic effects in MCF-7 human breast tumor xenografts in mice were evaluated.

2. Materials and methods

2.1. Materials

Sodium salt of hyaluronic acid (HA, molecular weight: 35 kDa, Shandong Freda Biopharm Co., Ltd., Shangdong, China), 1-(3-dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride (EDC·HCl, 98%, J&K, Beijing, China), N-hydroxysuccinimide (NHS, 98%, Alfa Aesar, USA), cystamine dihydrochloride (Cystamine·2HCl, >98%, Alfa Aesar, USA), *N*, *N*-dimethylformamide (DMF, anhydrous, >99.7%, Alfa Aesar), paclitaxel (PTX, >99%, Beijing ZhongShuo Pharmaceutical Technology Development Co., Ltd., Beijing, China), iodixanol (99.3%, Hubei Ju Sheng Technology Co., Ltd., Hubei, China), methacrylic anhydride (MA, 94%, J&K, Beijing, China), 4-dimethylamino pyridine (DMAP, 99%, Alfa Aesar, USA), dibutyltin dilaurate (DBTDL, 97.5%, J&K, Beijing, China), acetonitrile (99.3%, HPLC grade, Merck, Germany), glutathione (GSH, >98%, Amresco, USA), dithiothreitol (DTT, 99%, Merck, Germany), cyanine 5 (Cy5, 98%, Lumiprobe, USA), goat serum (Roche, Germany), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA), DAPI (Invitrogen, USA), trypsin (Jinuo Biomedical Technology, Hangzhou, Zhejiang, China), rat monoclonal *anti*-mouse CD31 (BD Pharmingen, San Jose, California, USA), Alexa 594 conjugated donkey anti-rat secondary antibody (Molecular Probes, Eugene, OR, USA), cell culture dishes and 24 and 96-well plates (Thermo Fisher Scientific, USA) were used as received. Pyridine, diethyl ether, dichloromethane (DCM), dimethyl sulfoxide (DMSO), *N*, *N*-Dimethyl formamide (DMF), and methanol were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Tetrazole (Tet) and cystamine diisocyanate (CDI) were synthesized according to our previous reports [36,42].

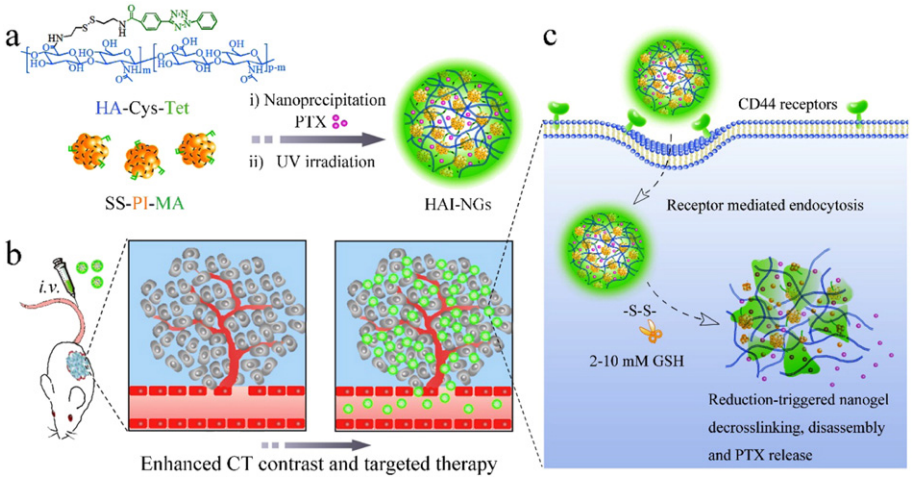
2.2. Cell culture and animal studies

MCF-7 breast cancer cells and L929 murine fibroblastic cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in DMEM medium (HyClone, Logan, Utah, USA) supplemented with 1% penicillin, 1% streptomycin (Jinuo Biomedical Technology, Hangzhou, Zhejiang, China), and 10% fetal bovine serum (FBS, Gibco, Invitrogen, USA). The cells were cultured as a monolayer in a humidified atmosphere containing 5% CO_2 at 37 °C.

Female Balb/c nude mice of 4-6 weeks age were purchased from Model Animal Research Center of Nanjing University. Mice were housed at 25 °C and 55% humidity under natural light/dark conditions and allowed free access to standard food and water. All animal procedures were performed following the protocol approved by the Animal Study Committee of Soochow University.

2.3. Synthesis of reductively degradable polyiodixanol-methacrylate (SS-PI-MA)

SS-PI-MA was readily synthesized *via* polyaddition of iodixanol and cystamine diisocyanate (CDI) followed by treatment with methacrylic anhydride (MA). Briefly, in a glove-box under a nitrogen atmosphere, iodixanol (500 mg, 0.32 mmol) was dissolved in anhydrous DMF



Scheme 1. Illustration of bioresponsive and fluorescent hyaluronic acid-iodixanol nanogels for targeted X-ray computed tomography imaging and chemotherapy of breast tumors. (a) PTX-loaded HAI-NGs are prepared *via* nanoprecipitation followed by crosslinking *via* UV irradiation; (b) PTX-loaded HAI-NGs actively target and accumulate at MCF-7 tumors, resulting in enhanced CT contrast and targeted therapy; (c) PTX-loaded HAI-NGs are selectively internalized into the MCF-7 breast tumor cells *via* CD44 receptor-mediated endocytosis, nanogels are decrosslinked and disassembled in response to GSH in the cytosol, and PTX is quickly released into the cells.