Materials Science and Engineering C 67 (2016) 159–169

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

A pH-sensitive hyaluronic acid prodrug modified with lactoferrin for glioma dual-targeted treatment

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ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 30 March 2016
Accepted 5 May 2016
Available online 06 May 2016

Keywords:
Lactoferrin
Hyaluronic acid
Glioma
Prodrug
Doxorubicin
Targeted treatment

ABSTRACT

Gliomas are the most common and lethal type of primary malignant brain tumor. But the existence of blood brain barrier (BBB) and blood-tumor barrier (BTB) hinder drug from reaching the tumor site. To address this problem, we developed a novel prodrug (Lf-HA-DOX) by conjugating hyaluronic acid with doxorubicin (HA-DOX) by an acid-labile hydrazone linkage, which is released in an acidic environment and accumulates in tumor tissues. Lactoferrin (Lf) was coupled for transporting across the BBB. In vitro, the release of DOX from Lf-HA-DOX was pH-dependent. At lower pH (5.0 and 6.0), the release of DOX was much quicker than that at pH 7.4. In the cellular uptake studies, flow cytometry analyses and confocal laser scanning microscopy results showed significantly enhanced cellular uptake of Lf-HA-DOX and HA-DOX in C6 cells compared to DOX. In BALB/C mice bearing C6 glioma, enhanced accumulation of Lf-HA-DOX in the glioma was observed by real time fluorescence image. Correspondingly, glioma-bearing mice treated with Lf-HA-DOX displayed the longest median survival time, which was 2-fold longer than that of saline group. In conclusion, Lf-HA-DOX was able to significantly increase drug delivery to the glioma, which might provide a promising strategy for antiglioma therapy.

1. Introduction

Doxorubicin (DOX) is an anthracycline that displays a broad spectrum of antitumor activity [1,2]. However, the clinical application of anthracyclines, including DOX, epirubicin, idarubicin, and daunorubicin is limited by their dose-related side effects. The most significance is its cumulative cardiotoxicity, which is irreversible and can lead to cardiomyopathy and congestive heart failure [3]. To minimize the toxic side effects of DOX, alternative dosage forms have been suggested, including liposomes [4–6], emulsions [7,8], and polymeric nanoparticles [9–11]. Another way is coupling of DOX to macromolecular carriers or water soluble polymers [12]. Although these new strategies improve the water solubility of the drug and reduce some of its dose-related side effects, they cannot overcome the problem that DOX lacks of the ability to penetrate through the blood-brain barrier (BBB) and release the active drug in brain gliomas.

Gliomas are the most common and invasive primary malignant brain tumors in adults [13]. Due to the infiltrative nature and resistant to radiation and chemotherapy, glioma can be hardly cured completely by conventional therapies, such as surgery, radiotherapy or chemotherapy [13,14]. The failure of the traditional chemotherapy is due to the existence of BBB and blood-brain tumor barrier (BBTB). BBB is a natural barrier which separates blood from the cerebral tissue, and BBTB is formed between the capillary and brain tumor [1,15]. These two barriers prevent almost all large molecule drugs and more than 98% small molecule candidate drugs penetration into the central nervous system, and form a major obstacle in brain tumor therapy [16–18].

Abbreviations: ADH, adipic acid dihydrazide; ANOVA, one-way analysis of variance; ATCC, American Type Culture Collection; BBB, blood brain barrier; BTB, blood-tumor barrier; CCK-8, Cell Counting Kit-8; DMEM, Dulbecco's modified Eagle's medium; DOX, doxorubicin; EDCI, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride; FBS, fetal bovine serum; HA, hyaluronic acid; HA-ADH, adipic acid dihydrazide modified HA; HE, hematoxylin and eosin; HOBt, 1-hydroxybenzotriazole; Lf, lactoferrin; LfR, Lf receptor; LRP, low-density lipoprotein receptor-related protein; MR, magnetic resonance; NHS, *N*-hydroxysuccinimide; PBS, phosphate buffer saline; RMT, receptor-mediated transcytosis; SD, standard deviation.

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http://dx.doi.org/10.1016/j.msec.2016.05.012
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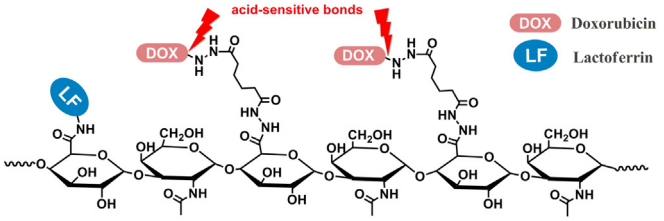

Fig. 1. Scheme of molecular structure of LF-HA-DOX prodrug.

It is known that there are several transport routes to move across the BBB [19,20], such as simply diffuse, receptor-mediated transcytosis (RMT), adsorptive-mediated transcytosis (AMT), etc. Among these transport routes, RMT provides an opportunity for selective uptake of macromolecules and active targeting of BBB [21]. AMT mainly provides the opportunity for albumin and other plasma proteins. Kamali M, et al. [22] has prepared the imatinib base loaded human serum albumin (HSA) and found that its cytotoxic effect is higher than that of free imatinib for glioblastoma. Although AMT enables many poorly brain-penetrating drugs across the BBB, yet AMT is a non-specific process. So the process will not only occur at the BBB but also in the blood vessels in other organs [21]. It is known that endothelial cells have many receptors (such as: transferring receptor, insulin receptor, lipoprotein receptors), to take in many different types of ligands, including enzymes and plasma proteins [23]. Lactoferrin (Lf) is an iron binding glycoprotein in the transferrin family [24,25]. The low-density lipoprotein receptor-related protein (LRP), the Lf receptor (LfR), has been proved overexpressed not only on the BBB, but also on the glioma cells surface [21,26–29]. So Lf here not only served as a promising targeting ligand to overcome the problem of effective penetration through the BBB, but also contributed to the glioma-targeted effect.

Hyaluronic acid (HA) is a principal ligand for the CD44 receptor which is crucial to cancer cell aggregation, proliferation, migration, and cancer metastasis [30]. High levels of CD44 are expressed in many cancers [31], such as liver carcinoma, glioma, etc. Recently, we have reported a HA-conjugated iron oxide nanoparticles for targeted MR imaging of liver carcinoma [32]. So HA can be used to target the glioma. Furthermore, glioma has the tumors' common properties, such as low pH, low pO2 [33–35]. It is known that endosomal pH within the tumor cells is significantly lower than that of the normal cells [36]. This property also be exploited by coupling DOX to HA-based delivery system through acid-sensitive hydrazone linkage in the present study.

In this study, we aimed at developing a dual-targeting drug delivery system. As illustrated in Fig. 1, DOX and HA were bound by an acid-labile hydrazone linkage, and then Lf was coupled with HA. The system, Lf-HA-DOX, was evaluated by its pH-responsive release. Here, we also evaluated the therapeutic efficacy of Lf-HA-DOX formulation against glioma cells. Flow cytometry (FCM) and confocal laser scanning microscopy (CLSM) were used to evaluate the cellular binding and uptake of Lf-HA-DOX formulation. Using the mice bearing C6 glioma cells, we assessed the therapeutic potential of Lf-HA-DOX compared to HA-DOX.

2. Materials and methods

2.1. Materials

HA (MW 6.4 kDa) was purchased from Freda Biochem Co., Ltd. (Shandong, China). 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide

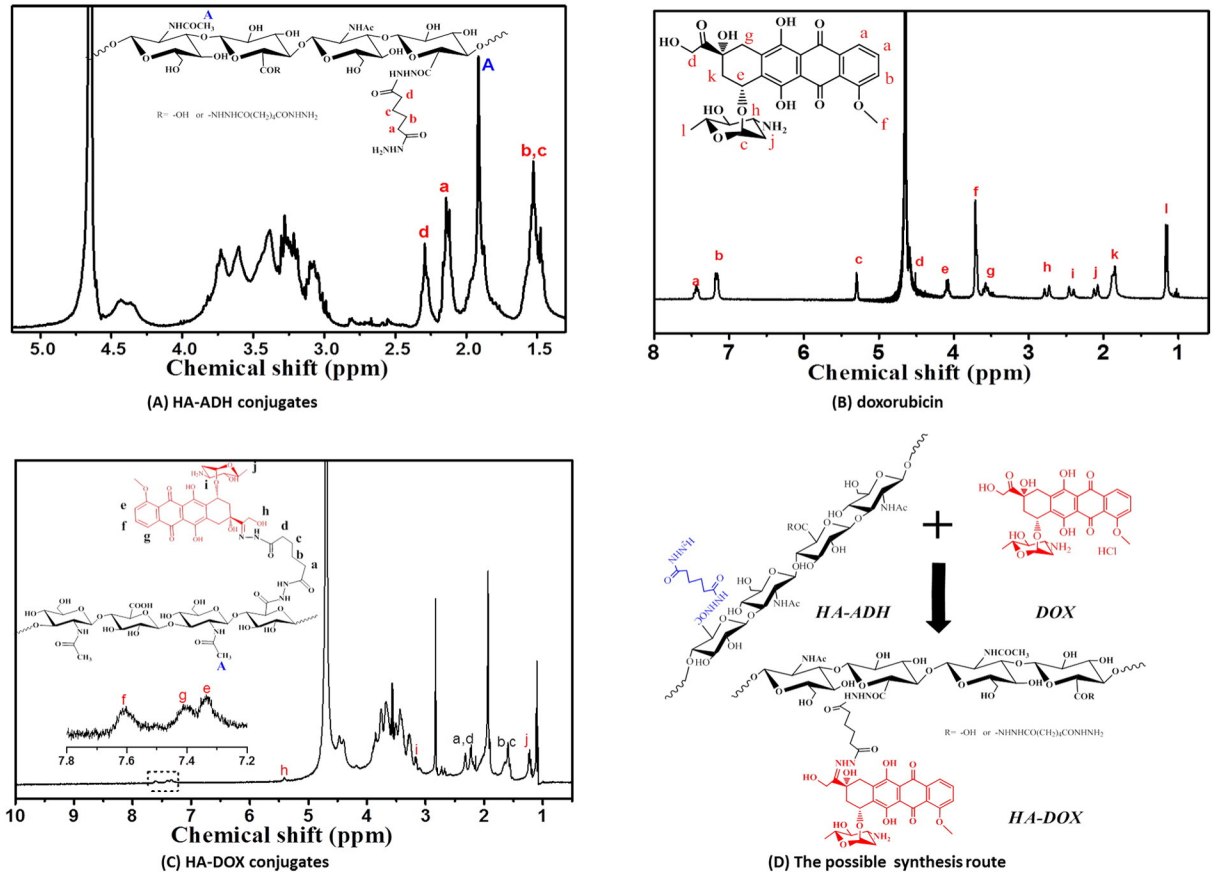

Fig. 2. ^{1}H NMR spectra of HA-ADH, doxorubicin and the conjugate prodrug (HA-DOX) in D2O.