Journal of Controlled Release 258 (2017) 56–66

Contents lists available at ScienceDirect

Journal of Controlled Release

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

cRGD peptide-installed epirubicin-loaded polymeric micelles for effective targeted therapy against brain tumors

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ARTICLE INFO

Keywords:
Polymeric micelle
pH-sensitivity
Epirubicin
Brain tumor
Targeted therapy

ABSTRACT

Current therapeutic strategies against glioblastoma multiforme (GBM) are futile mainly because of the poor access of drugs into malignant tissues, which is hindered by the tight blood-brain tumor barrier in the GBM vasculature. Nanomedicines have shown potential for circumventing the vascular barriers of GBM, particularly by targeting markers on the luminal side of endothelial cells in the blood vessels of GBM for achieving effective and selective translocation into the tumor. Thus, as the αvβ3 and αvβ5 integrins overexpressed on the endothelial cells of GBM can be targeted by cyclic-Arg-Gly-Asp (cRGD) peptide, herein, we developed cRGD-installed micellar nanomedicines loading epirubicin, the potent antiglioblastoma agent, through a pH-sensitive hydrazone-bond for effective treatment of GBM. These cRGD-installed epirubicin-loaded polymeric micelles (cRGD-Epi/m) achieved faster and higher penetration into U87MG cell-derived 3D-spheroids than the micelles without cRGD, conceivably through a cRGD-integrin mediated pathway. *In vivo*, the cRGD-installed micelles effectively suppressed the growth of an orthotopic GBM model by delivering high levels of epirubicin throughout the tumor tissue. These results indicate significant prospects for cRGD-Epi/m as an effective and translationable treatment against GBM.

1. Introduction

Glioblastoma multiforme (GBM) represents the most frequent and deadliest of astroglial tumors, with < 5% of patients with GBM surviving longer than 3 years [1,2]. The most dominant factors for this poor prognosis of GBM are its high invasive [3] and angiogenic [4] character, the inherent resistance of GBM to traditional therapies [2] and the presence of the blood-brain tumor barrier (BBTB) in the vasculature of GBM, which limits the penetration and accumulation of drugs [5,6]. The current standard treatment of GBM consists of maximal surgical resection followed by radiotherapy (RT) with concomitant and adjuvant chemotherapy with temozolomide (TMZ) [7]. While TMZ has made an impact on survival (median survival improved from 12.1 months with only RT to 14.6 months with RT plus TMZ); tumor recurrence and TMZ resistance remain major challenges [7,8]. Thus, effective chemotherapeutic approaches against GBM should be designed for overcoming the vascular barriers and being sufficiently potent to eradicate cancer cells.

Nanomedicines could improve the efficiency of chemotherapies by selectively delivering drugs to tumors [9–11]. While the preferential accumulation of nanomedicines in solid tumors is mediated by the enhanced permeability of the malignant vasculature [12], nanomedicines targeting GBM should be modified with ligands facilitating their translocation from the vasculature into the brain tumors [13,14], as the BBTB restricts their extravasation. Among nanomedicines with potential for clinical translation, drug-loaded polymeric micelles have demonstrated significant tumor targeting in both pre-clinical and clinical trials [15,16], and the modification of their surface with cyclic Arg-Gly-Asp (cRGD) peptides, which preferentially binds to αvβ3 and αvβ5 integrins [17–20] overexpressed in tumor neovasculature and tumor cells [21], including GBM cells [22–24], could facilitate their transcellular transport into a GBM model [25]. Thus, installation of cRGD peptides on clinically evaluated polymeric micelles incorporating potent drugs against GBM cells could provide substantial therapeutic

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http://dx.doi.org/10.1016/j.jconrel.2017.04.033
Received 29 December 2016; Received in revised form 12 April 2017; Accepted 24 April 2017
Available online 05 May 2017
0168-3659/

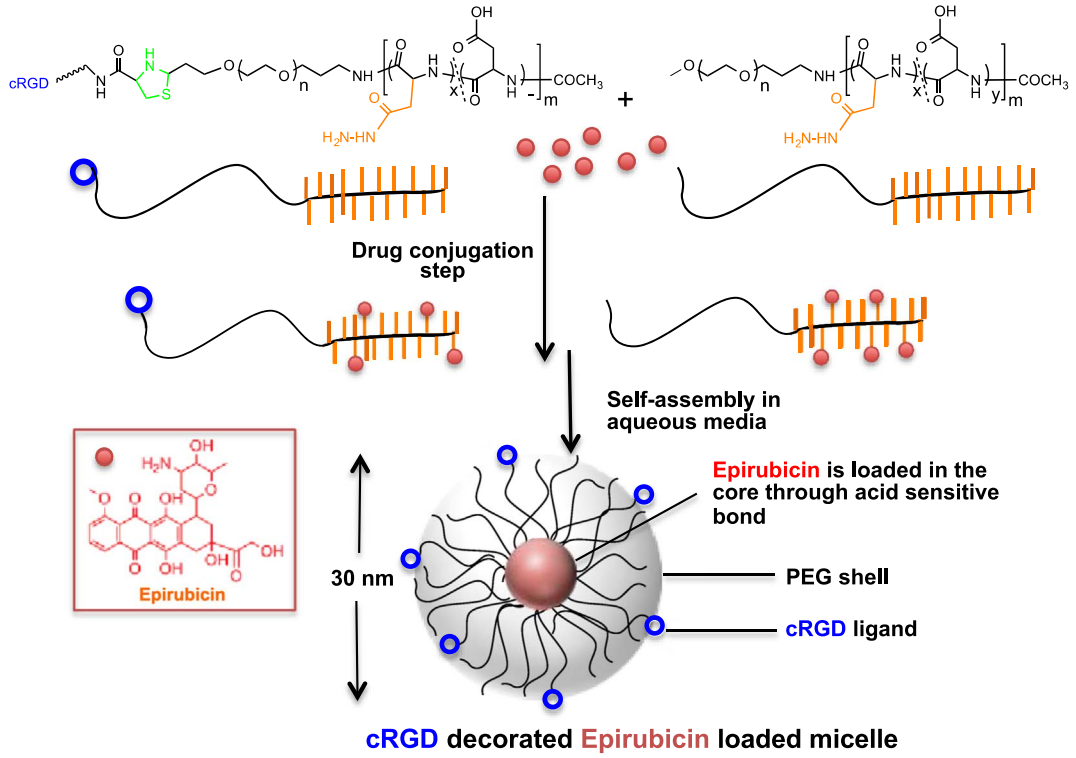

Fig. 1. Preparation of cRGD-peptide installed epirubicin-loaded polymeric micelles from a mixture (1:3) of cRGD-poly(ethylene glycol)-*b*-poly(hidrazinyl-aspartamide) and MeO-poly (ethylene glycol)-*b*-poly (hidrazinyl-aspartamide) polymer.

improvement and meet the urgent clinical needs.

Recently, epirubicin (Epi), an anthracycline, has been identified as one of the most potent antiglioblastoma agents from the NIH Clinical Collection (NCC) library of 446 FDA-approved drugs [26]. In fact, Epi was significantly more effective than current clinical antiglioblastoma drug TMZ [26]. However, anthracyclines fail to achieve therapeutic concentrations in brain tumors after intravenous injection due to their poor penetration through BBB/BBTB and efflux mediated by P-glycoprotein overexpressed in the endothelial cells of the BBB/BBTB [27]. Auspiciously, we have developed micellar nanomedicines loaded with Epi and their analogues by conjugating the drug to the poly(aspartic acid) segment of PEG-*b*-poly(aspartic acid) copolymers via an acid-sensitive hydrazone bond [28–30], which allows selective intracellular drug release after endocytosis. The Epi-loaded micelles (Epi/m) demonstrated low toxicity and high efficacy against several tumor models, and have now progressed into phase I human clinical trials (NC-6300; Nanocarrier Co., Ltd.) [16,29]. Herein, we have modified the surface of Epi/m with cRGD peptides aiming for effective GBM treatment (Fig. 1). Combining the pH-sensitive release mechanism with enhanced recognition and internalization by αvβ3/αvβ5 integrin targeting, these cRGD-installed Epi/m (cRGD-Epi/m) are expected to provide efficient antitumor effects against GBM tumor.

2. Materials and methods

2.1. Materials

Sources of materials for polymer synthesis and also basic cell culture reagents have already been described in our previous publication [31]. Additionally α-Acetal-ω-amino poly(ethylene glycol) (Acetal-PEG-NH_2; M_w = 12,000) was obtained from NOF Co, Inc. (Tokyo, Japan). Epirubicin was purchased from NanoCarrier (Chiba, Japan). Centrifugal filter tubes (MWCO = 30,000 Da) were purchased from Millipore Corporation. HPLC grade acetonitrile was purchased from Sigma-Aldrich Co. (St. Louis, MO). MTT in solid form was purchased from Dojindo Laboratories (Kumamoto, Japan). D-Luciferin potassium salt was bought from Promega Corporation.

2.2. Instruments related to polymer synthesis and characterization

For the description of the instruments for polymer synthesis and characterization we would like to refer to our previous publication [31].

2.3. Reversed-phase liquid chromatography instrumentation details

Reversed-phase liquid chromatography was performed at 40 °C on a Tosoh TSK-gel ODS-80TM (4.6 μm, 150 mm) with a Tosoh ODS-80TM guard cartridge (Tokyo, Japan). Detection was carried out using a Waters fluorescence detector with excitation and emission wavelengths of 488 and 560 nm, respectively.

2.4. Synthesis of Acetal-PEG-PBLA and MeO-PEG-PBLA

Acetal-poly(ethylene glycol)-*b*-poly(β-benzyl-aspartamide) (Acetal-PEG-PBLA) was synthesized using acetal-PEG-NH_2 as an initiator following a previously reported procedure [31,32]. The degree of polymerization was determined as 35 by ^{1}H NMR spectra using integral values from the $-CH_2CH_2O-$ proton peaks of PEG (MW = 12,000) and aryl (C_6H_5-) peak of PBLA block.

Similar procedure was used to prepare MeO-PEG-PBLA.

2.5. Acetylation of the terminal amine function of Acetal-PEG-PBLA

Acetylation of the terminal amine function of Acetal-PEG-PBLA was done according the literature procedure [31]. The efficiency of acetylation at the terminal amine was confirmed by ^{1}H NMR spectra using integral values from the methyl proton (2 × 3) peaks of acetal group (1.08 ppm) and acyl (CH_3CO-) peak (1.75 ppm) and two other methylene peak of the polymer (1.55 and 1.7 ppm).