Cancer Letters 400 (2017) 1–8

Contents lists available at ScienceDirect

Cancer Letters

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

Original Article

Chitosan-modified PLGA nanoparticles tagged with 5TR1 aptamer for *in vivo* tumor-targeted drug delivery

Sahar Taghavi [a], Mohammad Ramezani [a], Mona Alibolandi [a], Khalil Abnous [a, b, *], Seyed Mohammad Taghdisi [c, d, **]

[a] Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Targeted Drug Delivery Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form
10 March 2017
Accepted 5 April 2017

Keywords:
Epirubicin
PLGA
5TR1aptamer
Chitosan

ABSTRACT

In this study, we reported epirubicin (Epi) encapsulated nanoparticles (NPs) formulated with biocompatible and biodegradable poly (lactic-co-glycolic acid) (PLGA) modified with chitosan (CS) through a physical adsorption method. Using chitosan, the solubility and surface charge of PLGA was modified to make efficient drug carriers for cancer cells. To improve the anti-tumor efficacy, we developed targeted therapy of tumor cells using a 5TR1 DNA aptamer (Apt) against the MUC1 receptor. To prove the MUC1 receptor-mediated uptake of Epi-PLGA-CS-Apt NPs in the cells, competition experiments were carried out.

In vitro experiments, cytotoxicity assay and fluorescence uptake assay demonstrated that fabricated NPs with or without aptamers showed significantly high therapeutic efficiency in MCF7 cells (breast cancer cell) compared with free Epi, while in BALB/c mice bearing C26 cells (murine colon carcinoma cell), targeted NP groups exhibited significant tumor growth inhibition and higher inclination to tumor compared with non-targeted NPs. Hence, our *in vivo* results revealed that non-targeted NPs may diffuse away from the tumor site and release Epi in the extracellular space and decrease concentration of the drug in the targeted tissue. This study indicated Epi-PLGA-CS-Apt has great potential as a promising nanoplatform for *in vivo* cancer therapy and could be of great value in medical use.

Introduction

Anthracyclines are the most effective anti-tumors among all classes of chemotherapeutic agents. Epirubicin (Epi) is a widely used anthracycline in breast cancer chemotherapy [1,2]. Epi intercalates to DNA and inhibits topoisomerase II thereby leading to cell death [1,3]. Even though chemotherapy is still the frontline treatment strategy for cancers, its side effects, including effects on normal cells and limited treatment duration or dosing, reduce clinical applications [4,5]. Today, nanotechnology is paving the way to overcome the many barriers for efficient drug delivery [6]. A favorable approach to advance the selectivity and specificity of drugs for tumor cells is targeted drug delivery using nanoparticle functionalized with targeting ligands [4,7,8].

Choice of tumor marker and tumor-targeting ligand are important elements for targeted cancer therapy. Development of targeting ligands could reduce the off-target effects of drugs and cause them to be accumulated at the site of action [2]. The novel generation of targeting ligands is aptamers.

Aptamers are a class of nucleic acid-based ligands that fold into well-defined 3D structures to specifically bind to their targets [9,10]. Low toxicity, slow degradation kinetics and notable stability in a wide range of pH, temperature and organic solvents without loss of activity make aptamers powerful candidates for pharmaceutical applications [11–13]. These ligands also play a critical role in the development of novel biosensors [14].

Overexpression of a specific maker on the cell surface is used to justify cell-specific targeted drug delivery [15]. Mucin 1 (MUC1) is a cell surface glycoprotein with extensive glycosylated extracellular

* Corresponding author. Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Fax: +98 513 882 3255.
** Corresponding author. Targeted Drug Delivery Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Fax: +98 513 882 3255.
E-mail addresses: abnouskh@mums.ac.ir (K. Abnous), taghdisihm@mums.ac.ir (S.M. Taghdisi).

http://dx.doi.org/10.1016/j.canlet.2017.04.008
0304-3835/

domain [16]. Striking overexpression of the MUC1 receptor on the surface of tumor cells suggested that 5TR1 (Apt), anti-MUC1 aptamer would be suitable for tumor targeting [17,18].

The development of targeted drug delivery systems with nanoparticles is expected to have a big impact on the clinical approaches in tumor chemotherapy [19,20]. So far, a few aptamer-based targeted nanosystems have been introduced for treatment of breast and colon cancers. However, great attempts are being devoted to develop targeted delivery systems with better function and characteristic. One of these targeted delivery systems for treatment of breast and colon cancers has been introduced previously by our group. In this work, an aptamer-based dendrimer nanostructure was presented for targeted delivery of Epi to cancer cells [21]. The dendrimer structure was composed of several DNA building blocks. Although the developed delivery system showed good function in treatment of cancer cells *in vitro* and *in vivo*, the design of DNA origamis like this structure is complicated, and because of the presence of several DNA building blocks, they have a high cost. Thus here, we developed a targeted delivery system which did not have these shortcomings and has a simple design, low cost, more Epi loading and lower drug release in peripheral circulation.

Drug delivery systems using biodegradable and biocompatible nanoparticles, which have been approved by the Food and Drug Administration (FDA), like poly (lactic-co-glycolic acid) (PLGA), are of interest as they can be easily extended to clinical trials [5,22,23]. PLGA is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA), and its breakdown products are hydrophilic, diffusible and rapidly metabolized in the human body [24]. Nevertheless, the short residence time of this nanosystem represents a major limitation of PLGA for achieving effective drug targeting to the site of action [6,25]. Thus, to maximize the therapeutic effects of these carriers, they should bypass the phagocytic effects via surface coating of PLGA with hydrophilic polymers such as chitosan (CS) [6]. Chitosan is a biodegradable and biocompatible cationic polymer with relatively low immunogenicity. Because of its unique properties, chitosan has been widely used as a vehicle in drug delivery systems [26–28]. Also, the presence of primary amino groups on the chitosan polymer backbone could facilitate its attachment to other chemical reagents [24].

The present study is aimed at developing a targeted delivery system using modified PLGA nanoparticles with the chitosan and MUC1 aptamer. MCF7 and CHO cell lines were chosen to evaluate the effects of the designed delivery system *in vitro*. Consequently, using mice bearing C26 colon carcinoma, we assessed therapeutic efficacy of fabricated NPs with or without aptamers.

Materials and methods

Materials

The MUC1 aptamer (5TR1) (GAAGTGAAAATGACAGAACACAACA) was obtained from Bioneer (South Korea). MCF7 (C135, breast cancer cell) and CHO (C111, Chinese hamster ovary cell) cell lines were purchased from Pasteur Institute of Iran, and C26 cells (murine colon carcinoma cell) were obtained from Cell Lines Service (Eppelheim, Germany) and cultured in RPMI 1640 and 10% fetal bovine serum (Gibco, Gaithersburg, USA). DNase RNase-free water was purchased from Sigma. Epirubicin (Epi), chitosan (CS, medium viscosity; deacetylation degree, >84%), polyvinyl alcohol (PVA, with 86.7–88.7% hydrolysis degree and molecular mass 31 k) and PLGA-COOH with a lactide to glycolide ratio of 50/50 and an average molecular weight of 7–17 k were obtained from Sigma–Aldrich (USA). Methylene chloride (DCM) was provided by Merck.

Preparation of chitosan-modified PLGA encapsulated Epi nanoparticles

The surface of Epi loaded PLGA nanoparticles was modified with chitosan (Epi-PLGA-CS) through non-covalent electrostatic adsorption, as chitosan and PLGA are positively and negatively charged, respectively. The nanoparticles were prepared by a modified aqueous drug solution (W1)/organic phase (O)/external phase (W2) multiple emulsion and solvent evaporation technique. Briefly, 100 mg PLGA was first dissolved in 6 mL organic solvent (DCM) (O). 3 mL Epi (2 mg/mL) (W1) was added to the organic phase and emulsified using a probe sonicator (Hielscher, UP200Ht) for 2 min with a 30% amplitude to prepare W1/O emulsion. Chitosan solutions were prepared by dissolving 40 mg chitosan in 2 mL acetic acid (1%) at room temperature. Also, a PVA solution (5% w/v) in double distilled water was prepared and added to the chitosan solution as a surfactant (W2). The first emulsion was added to the W2 and sonicated for 1 min. The pH of outer aqueous phase was increased to 7.0, and then the reaction process was continued under constant magnetic stirring (200 rpm) at room temperature until the solvent evaporated. To remove the Epi adsorbed on the surface of particles as well as excessive surfactant, nanoparticles were washed three times with distilled water. Both supernatants and filtrates from the wash steps were collected for future analysis. Final products were obtained after lyophilization of the aqueous solution.

Loading of Epi into the nanoparticles

To determine the percentage of Epi entrapped in PLGA nanoparticles, we measured the drugs that were not encapsulated and were free in the supernatant (Eq. (a)). A fluorescence spectra of collected supernatant was obtained by Synergy H4 hybrid reader [$\lambda_{Ex} = 488$ nm, $\lambda_{Em} = 530–700$ nm] (BioTek, USA). Then a standard fluorescence curve was plotted with a series of standard Epi concentrations. Epi loading efficiency (%) and Epi loading content (%) of the prepared formulation were calculated by Eqs. (b) and (c), respectively.

$$\text{Mass of Epi within nanoparticles} = \text{Epi total} - \text{Epi in supernatant} \quad \text{(a)}$$

$$\text{Epi loading Efficiency}(\%) = \frac{\text{Mass of Epi within the nanoparticle}}{\text{Mass of Epi initially used}} \times 100 \quad \text{(b)}$$

$$\text{Epi loading content}(\%) = \frac{\text{Mass of Epi within the nanoparticle}}{\text{Mass of nanoparticle}} \times 100 \quad \text{(c)}$$

Conjugation of 5TR1 on the surface of nanoparticles

For aptamer conjugation studies, 5TR1 aptamer was electrostatically coupled to nanoparticles. Suspension of 1 mg/mL nanoparticles was prepared in DNase RNase-free water and sonicated for 30 min. After sonication, the suspension was centrifuged at 10,000 rpm for 10 min. Then, the supernatant was collected and stirred with 10 μL aptamer (100 nM) for 2 h.

Particle size analysis and zeta potential measurement

Mean particle size and zeta potential of the prepared nanoparticles with or without aptamer were determined using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, UK). Also, particle size of the nanoparticles with or without aptamer was confirmed by atomic force microscopy (AFM) (JPK Nano-Wizard II, Germany).

Epi release from the nanoparticles

The *in vitro* drug release from Epi-PLGA-CS nanoparticles was measured in triplicate in phosphate buffered saline (PBS) in pH 7.4 and citrate buffer in pH 5.5 by an equilibrium dialysis method. Suspension of 10 mg fabricated NP (400 μg Epi equivalent) in 4 mL 0.1 M PBS buffer (pH 7.4) or citrate buffer (pH 5.5) was sealed in a dialysis bag with a molecular weight cut-off (MWCO) of 10 kDa and immersed in 50 mL of the same buffer solution and incubated at 37 °C with gentle shaking. At specific time intervals, 1 mL of outer phase was removed for analysis and replaced by adding 1 mL fresh buffer with the same pH to the dialysis medium. The amount of released Epi was calculated by determining the absorbance of the supernatant at 488 nm at selected time intervals.

Cellular uptake of nanoparticles

Cellular uptakes of Epi-PLGA-CS, Epi-PLGA-CS-Apt and Epi were recorded using a fluorescence microscope (CETI Microscopes, UK). MCF7 cells were seeded into a 6-well plate at a density of 12×10^4 cells per well in 2 mL of culture medium. After overnight incubation, the medium was replaced with 2 mL of fresh medium containing free Epi (7 μM) or prepared formulations with equal concentration of Epi (7 μM). After 2 h of incubation, in the presence of nanoparticles, the cells were washed with PBS and the medium was replaced with a nanoparticle-free fresh medium. Images were taken with fluorescence microscope. To prove the targeted delivery of Epi-PLGA-CS-Apt, 8 μg/well excess of 5TR1 aptamer was added to the culture medium and incubated for 30 min. Then other steps were carried out as described above.

MTT assay

The cytotoxic effect of Epi was evaluated using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] colorimetric assay, which is a quantitative and rapid colorimetric method based on the cleavage of a yellow tetrazolium salt to insoluble purple formazan crystals by the mitochondrial