Carbohydrate Polymers 201 (2018) 131–140

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Glutathione responsive chitosan-thiolated dextran conjugated miR-145 nanoparticles targeted with AS1411 aptamer for cancer treatment

Farnaz Sadat Mirzazadeh Tekie[a,b], Masoud Soleimani[c,d], Amir Zakerian[c], Meshkat Dinarvand[a,e], Mohsen Amini[f], Rassoul Dinarvand[a,b], Ehsan Arefian[c,g], Fatemeh Atyabi[a,b,*]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box, 14155-6451, Tehran, Iran
[b] Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, P.O. Box, 14155-3174, Tehran, Iran
[d] Department of Hematology, School of Medical Sciences, Tarbiat Modares University, P.O. Box, 14115-111, Tehran, Iran
[e] Center of nanomedicine, Brigham and woman's hospital, Harvard Medical School, Boston, MA, 02115, USA
[f] Department of Chemical Medicine, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box, 14155-6451, Tehran, Iran
[g] Department of Microbiology, School of Biology, College of Science, University of Tehran, Iran

ARTICLE INFO

Keywords:
miR-145
Thiolated dextran
Chitosan
PEC
AS1411

ABSTRACT

miR-145 is a tumor suppressive miRNA which is abnormally reduced in different cancers. miR-145 over-expression reduces cancer migration, invasion, and cell adhesion. Increasing miR-145 level using suitable and efficient gene delivery systems could be valuable in cancer treatment. In this study, a redox-responsive miR-145 conjugated thiolated dextran (TD-miR) was prepared. Also, polyelectrolyte complexes (PECs) of TD-miR and chitosan were fabricated and decorated with anti nucleolin aptamer, AS1411 (apt-PEC). The size of the PECs was between 40–270 nm, and the zeta potential was varied according to the TD-miR to chitosan molar ratio. The outcomes of cellular studies indicated the excellence of the apt-PEC as a duel targeted delivery system and the PECs composed of chitosan 18 kDa with TD-miR to chitosan ratio of 5. TD-miR and the PECs are appropriate as the smart gene delivery systems which preserve and transfect the cargo and release it in cytoplasm.

1. Introduction

miRNAs are small endogenous non-coding RNAs regulate protein expression via triggering cleavage or translation suppression of mRNAs (Iorio, Casalini, Tagliabue, Ménard, & Croce, 2008). miRNAs have crucial role in development of cancers by controlling various cellular processes such as cell proliferation and differentiation (Meng et al., 2006). Expression profiles of two groups of miRNAs including oncogenic and tumor suppressive miRNAs are altered in cancers (Babashah & Soleimani, 2011). miR-145 is one of the aberrantly reduced miRNAs in various cancers and it functions as tumor suppressor by targeting MUC1, c-Myc, fascin-1, IGF-1R, Oct-4 and etc. (Kim et al., 2011; Sachdeva & Mo, 2010b). Accordingly, miR-145 is potentially useful for treatment of cancers. For instance, Ibrahim et al. demonstrated that miR-145 replacement therapy is efficacious in colon cancer (Ibrahim et al., 2011). In our previous study, we confirmed that regulating the miR-145 expression by a chitosan-mediated delivery system can reduce proliferation rate of MCF7 breast cancer cells (Tekie et al., 2015).

One of the challenges in gene therapy is providing appropriate vectors for efficient transferring of gene materials into target cells. Biocompatibility, non-toxicity, biodegradability, ability to preserving the gene from serum and cytoplasm nucleases, and high cellular uptake are the critical properties expected from appropriate vector. In recent years, various polymeric systems have been designed for delivery of genes and macromolecules. Chitosan, a cationic carbohydrate polymer, has been extensively studied for gene delivery (Pack, Hoffman, Pun, &

Abbreviations: miRNAs, micro RNAs; PU-PEI, polyurethane-short branch-polyethylenimine; PEI, polyethylenimine; siRNA, small interfering RNA; TD, thiolated dextran; L-Cys, L-cysteine; CMD, carboxymethyl dextran; GSH, glutathione; PEC, polyelectrolyte complexes; Mw, molecular weight; TD-miR, conjugate of TD and miR-145; EDTA, ethylenediaminetetraacetic acid; DTNB, 5,5-Dithiobis(2-nitrobenzoic acid); BSA, bovine serum albumin; FBS, heat inactivated fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; RT-PCR, reverse transcription polymerase chain reaction; MUC1, anti mucin1; FTIR, Fourier transform infrared; DTT, dithiothreitol; Ch9, chitosan 9 kDa; Ch18, chitosan 18 kDa; Rpm, round per minute; D/Ch, dextran to chitosan molar ratio; Cy5PECs, Cy5 labeled PECs; Apt-PEC, AS1411 aptamer modified PEC; DLS, dynamic light scattering; TEM, transmission electron microscopy; PDI, polydispersity index; SD, standard deviation; ANOVA, one-way analysis of variance; PBS, phosphate buffer solution; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbadiimide; NHS, N-hydroxysuccinimide

* Corresponding author at: Faculty of Pharmacy, Tehran University of Medical Sciences, Nanomedicine, Enghelab St., Teheran, Iran.
E-mail address: atyabifa@tums.ac.ir (F. Atyabi).

https://doi.org/10.1016/j.carbpol.2018.08.060
Received 29 May 2018; Received in revised form 4 August 2018; Accepted 15 August 2018
Available online 17 August 2018
0144-8617/ © 2018 Published by Elsevier Ltd.

Stayton, 2005). Conjugates have been also developed to improve the stability and biological efficacy of the siRNAs. The siRNA conjugates have been widely noticed in preclinical studies due to the advantages of this strategy over other delivery systems including simple and well-defined molecular structure of the conjugates and controllable formulation process. Nevertheless; in compare with particulate delivery systems, the conjugates have some cons such as fast renal exertion, lower stability against nucleases, poor extravasation and low accumulation in target sites (Jeong, Mok, Oh, & Park, 2009; Lee, Kang, Jang, & Mok, 2016).

Dextran is an FDA-approved poly-glucose biopolymer that is widely used in drug conjugation strategies due to its desirable physicochemical characteristics along with its low cost and a history of clinical usage (Goodarzi, Varshochian, Kamalinia, Atyabi, & Dinarvand, 2013; Yousefpour, Atyabi, Farahani, Sakhtianchi, & Dinarvand, 2011). Although polyanionic structure of dextran salts restricted its application in gene delivery, various dextran-based delivery systems with controlled release manner, such as cationic dextran particles, were designed for siRNA delivery (Cohen et al., 2011; Raemdonck et al., 2009).

Thiomers or thiolated polymers are mocuadhesive polymers have been used in oral drug delivery to enhance permeation of drug molecules (Atyabi, Talaie, & Dinarvand, 2009; Saremi, Atyabi, Akhlaghi, Ostad, & Dinarvand, 2011). Thiomers are also promising candidate for gene delivery. In our previous studies, we prepared nanoparticles composed of thiolated chitosan to transfer antisense oligonucleotide to the cancer cell lines. The results indicated the adequate efficiency of the thiomers in gene delivery (Dinarvand et al., 2015; Talaei, Azizi, Dinarvand, & Atyabi, 2011). Since disulfide bonds are reduced in response to the high level of intracellular GSH, conjugates of thiomers and pharmaceutical molecules by disulfide bonds have attracted scientists to develop redox-responsive systems for targeted intracellular drug and gene delivery (Cheng et al., 2011). In our prior study, we prepared nano PECs of TD/ CMD and chitosan for delivery of anti h-SET1 antisense oligonucleotide to the cancer cells. The antisense was physically entrapped into the nanoparticles and following entrance to the cells, it was released by reducing disulfide crosslinks between the polymer chains and dissociation of the complexes. Results confirmed the nobility of using TD in PEC structure due to the better physical characteristics, higher stability, mucoadhesion, and superior uptake of the thiolated PECs (Kiani et al., 2016).

PECs are the complex of chitosan and polyelectrolytes such as polysaccharides with opposite charge including dextran sulfate, carboxymethyl cellulose, and alginates. PECs are believed as the prosperous vehicles for biological macromolecules because of a simple and safe preparation method in aqueous media, non-toxicity, and protective properties (Liu, Jiao, Wang, Zhou, & Zhang, 2008).

In our prior study, it was indicated that optimizing CMD and chitosan PEC preparation procedure and structural factors such as Mw and the molar ratio of the polymers led to the nanoparticles with adequate size distribution, loading, and transfection efficiency because appropriate balance between stability and dissociation rate of the complex in serum contained cell culture medium was achieved (Tekie et al., 2017).

One of the approaches for improving nanoparticle efficiency is using targeting agents such as aptamers. Aptamers are short RNA or single-stranded DNA sequences that by their distinctive 3D conformation could bind to the targets with high specificity and affinity (Sun & Zu, 2015). AS1411 aptamer is the 26-nucleotide guanosine-rich DNA sequence which binds to the nucleolin receptors over expressed on cancer cells. Different studies verified that the AS1411 aptamer increases the uptake of nanoparticles in cancer cells such as MCF-7 (Shieh, Yang, Wei, & Shieh, 2010).

As carbohydrate polymers including dextran and chitosan are among the attractive biocompatible and biodegradable polymers which have been widely studied as drug and gene delivery system, herein we developed a novel and efficient dual targeted controlled release dextran-chitosan based gene vehicle.

2. Hypothesis

We purpose that the stability and efficacy of the miRNA will be enhanced by synthesis of TD and miRNA conjugate (TD-miR) via disulfide bond as the GSH responsive system in compare with non-conjugated miRNA. We also suggest that nano PECs of TD-miR and chitosan can further enhance the permeability and retention time of the miRNA significantly, which is the well-known advantage of nanoparticles. By this strategy, we can simultaneously benefit from the pros of conjugates and nanoparticles. This study also included attachment of the anti nucleolin aptamer (AS1411) as a targeting agent to enhance the uptake of nanoparticles by cancer cells in compare with non-targeted nanoparticles resulting in higher gene silencing and cancer cell apoptosis.

3. Materials and methods

3.1. Materials

It was presented in supporting information (S1).

3.2. Synthesis and characterization of TD

The synthesis of L-Cys conjugated CMD was performed according to the previously reported method with minor modification (G Shahnaz, Perera, Sakloetsakun, Rahmat, & Bernkop-Schnürch, 2010). CMD (60 g/l) was dissolved in distilled water (5 ml), and the carboxyl moieties were activated by adding EDC (150 mM). The reaction proceeded for about 45 min at room temperature. L-Cys (60 g/l) was added and pH was raised to 6 by sodium hydroxide 0.1 N. The reaction mixture was stirred under nitrogen blanket for 4 h at room temperature. The obtained polymer was purified using dialysis membrane (MWCO: 8 kDa) against 500 ml of HCL 5 mM for 6 h, HCL 5 mM and NaCl 1% for 12 h, HCL 5 mM for 6 h, and HCL 1 mM for 12 h. The structure and thiolation rate of the modified polymer were investigated using FTIR spectroscopy. The thiol substitution was also estimated by colorimetric reaction using Ellman's reagent, DTNB (Kiani et al., 2016).

3.3. Preparing of double strand miRNA-145

It was presented in supporting information S2.

3.4. Chitosan nitrous acid hydrolysis

Depolymerization of fully de-acetylated chitosan with the Mw of 400 kDa to the 9 kDa (Ch9), and 18 kDa (Ch18) was performed via nitrous acid hydrolysis (Tekie et al., 2015). The purified chitosan was characterized by viscometeric analysis as described before (Tekie et al., 2017).

3.5. Synthesis of dextran and miR-145 conjugate (TD-miR)

Synthesis method of TD-miR was developed following trying out various condition with different temperature, oxygen pressure, component concentration and pH which was presented in supporting information in details (S3). The selected method: 150 µl of TD stock solution (500 µg/ml) prepared in distilled water and 50 µl of miR-145 stock solution (150 µg/ml) in tris buffer (pH, 8) was mixed and incubated under light at room temperature for 12 h.

3.6. Preparing polyelectrolyte complexes

The PECs of TD-miR and chitosan were obtained by mixing adequate volume of TD-miR stock solution with chitosan in acetate buffer (pH 5.5). After diluting the mixtures up to 500 µl by acetate buffer (pH 5.5), they were stirred by vortex stirrer for 20–30 s (2500 rpm) and left

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