European Journal of Pharmaceutical Sciences xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Synthesis and characterization of cystamine conjugated chitosan-SS-mPEG based 5-Fluorouracil loaded polymeric nanoparticles for redox responsive drug release

M. Gover Antoniraj[a], S. Angelin Tisha[a], Ayyavu Mahesh[c], A. Shanmugarathinam[a], Ruckmani Kandasamy[a,b,*]

[a] Department of Pharmaceutical Technology, Centre for Excellence in Nanobio Translational Research (CENTRE), Anna University, BIT campus, Tiruchirappalli 620024, Tamil Nadu, India

[b] National Facility for Drug Development for Academia, Pharmaceutical and Allied Industries (NFDD), Anna University, BIT campus, Tiruchirappalli 620024, Tamil Nadu, India

[c] Centre for Excellence in Genomic Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai 625021, Tamil Nadu, India

ARTICLE INFO

Keywords:
Chitosan
Methoxy poly(ethylene glycol)
Cystamine dihydrochloride
Polymeric nanoparticles
Redox responsive drug release

ABSTRACT

The principle objective of this study was to develop and characterize redox responsive polymeric nanoparticles (PNPs) as a stimuli responsive drug delivery system. The chitosan-cystamine-methoxy poly(ethylene glycol) (CH-SS-mPEG) copolymer was synthesized by conjugation of cystamine appended chitosan with carboxylic acid-terminated mPEG and characterized by FTIR, ^{1}H NMR, XRD analysis and colorimetric assay. This copolymer could be formulated as 5-Fluorouracil (5-FU) loaded PNPs and the characteristics of PNPs were evaluated. Moreover, folic acid functionalized PNPs were prepared for folate receptor targeted drug delivery. Drug release studies indicated that the redox sensitive PNPs were stable in physiological condition while quickly releasing 5-FU in the trigger of redox potential due to the cleavage of the disulfide linkages. In contrast, less quantity of drug was released from the reduction insensitive chitosan-g-methoxy poly(ethylene glycol) (CH-g-mPEG) based PNPs under both reduction sensitive and non-reductive conditions. From the cytotoxicity studies, it was evident that 5-FU loaded PNPs had higher toxicity against MCF7 cells when compared to 5-FU free PNPs. Subsequently, cellular uptake studies showed significantly increased internalization of folic acid attached PNPs. In conclusion, the developed PNPs appeared to be of great promise in redox responsive drug release for targeted drug delivery.

1. Introduction

Challenges of the current day medicine demands for development of specialized drug delivery systems, especially for life threatening diseases, such as, ischemic heart disease, cancer and respiratory infections (Suarez et al., 2015; Upadhyay et al., 2015). In such concern, various nanocarriers such as polymeric micelles, liposomes, nanogels, nanospheres and polymer drug conjugates have been developed so far (Chacko et al., 2012; Prokop and Davidson, 2008). These nano-scale carriers are capacitated to passively accumulate in organ or in tissues by enhanced permeation and retention (EPR) effect and increased therapeutic efficiency (Chen et al., 2014; Markman et al., 2013; Prokop and Davidson, 2008). To further improve the spatial and temporal drug delivery, much research effort has been directed for the development of stimuli-sensitive drug delivery system that can facilitate the release of therapeutic moiety which is regulated by physiological stimulus (such as pH, temperature, glutathione, etc.) after reaching the disease site (Abulateefeh et al., 2013; Huang et al., 2013). Upon reaching the disease site, stimuli responsive drug delivery system is localized into cells and is consequently triggered by the stimuli to release the drug, thereby, inducing the required therapeutic activity in the diseased cells, with reduced side effects to non-targeted cells (Mura et al., 2013; Shim and Kwon, 2012). Among different stimuli responsive drug delivery system, redox responsive drug delivery has acquired much more attention due to the existence of high difference redox stimulus between normal and pathological sites. For example, higher concentration of

Abbreviations: 5-FU, 5-Fluorouracil; PNPs, polymeric nanoparticles; EPR, enhanced permeation and retention; CH-SS-mPEG, chitosan-cystamine-methoxy poly(ethylene glycol); CH-g-mPEG, chitosan-*graft*-methoxy poly(ethylene glycol); GSH, glutathione; mPEG, methoxy poly(ethylene glycol); MCF7, human breast adenocarcinoma cell line; TNBS, 2,4,6-Trinitrobenzenesulfonic acid; TPP, tripoly phosphate; FITC, Fluorescein isothiocyanate; RES, reticuloendothelial system; FA, folic acid; DLS, Dynamic light scattering

* Corresponding author at: Department of Pharmaceutical Technology, Centre for Excellence in Nanobio Translational Research (CENTRE), Anna University, BIT campus, Tiruchirappalli 620024, Tamil Nadu, India.

E-mail addresses: hodpharma@gmail.com, k.ruckmani@aubit.edu.in (R. Kandasamy).

http://dx.doi.org/10.1016/j.ejps.2017.10.035
Received 21 May 2017; Received in revised form 2 October 2017; Accepted 24 October 2017

glutathione (GSH) (2–10 mM) present in the normal intracellular environment than that in the extracellular environment (2–20 μM), due to enzymatic degradation in the extracellular environment, causes more redox potential in intracellular spaces (Sun et al., 2009; Wang et al., 2011; Wang et al., 2012). Moreover, extensive oxidative stress in the inflamed and tumor cells produce higher concentration of intracellular GSH, with respect to normal cells.

In this perspective, numerous studies have been carried out to develop stimuli responsive drug delivery that responds to redox stimuli for selective delivery of therapeutic agents. Sun et al. (2009) developed poly(ethylene glycol)-SS-poly(ε-caprolactone) based doxorubicin loaded micelles which showed redox responsive drug release. Cai et al. (2011) formulated DNA complexed mPEG-SS-PLL mediated non-viral vectors and evaluated their redox responsive behavior. Li et al. (2012) fabricated redox responsive micelles self-assembled from disulfide containing amphiphilic hyaluronic acid-SS-deoxycholic acid conjugate for intracellular delivery of paclitaxel. In the current study, chitosan and methoxy poly(ethylene glycol) (mPEG) polymers have been utilized for the synthesis of a disulfide-linked, redox stimulus responsive copolymer noted as chitosan-SS-methoxy poly(ethylene glycol) (CH-SS-mPEG).

Chitosan is a cationic linear polysaccharide derived from chitin by deacetylation and is considered as a good carrier in therapeutics as it is biocompatible, biodegradable and non-toxic. Due to its reactive amino and hydroxyl functional groups, chitosan has been extensively attracted by researchers for its effective utilization in the drug delivery, gene delivery and biomedical applications (Zhang et al., 2009).

mPEG is a highly hydrophilic and biocompatible synthetic polymer suitable for the generation of drug delivery carriers (Zhang et al., 2009; Malhotra et al., 2013). In addition, the simplicity of synthesis of different derivatives of mPEG capable of formation of graft copolymers with different kind of polymers and copolymers have been mostly utilized in various drug delivery applications (Zalipsky, 1995).

Chitosan and mPEG based copolymers have been extensively reported for drug delivery applications. In recent years, chitosan and mPEG based copolymers have been investigated for reduction-sensitive drug delivery. However, the general methods followed for synthesis of disulfide bond containing copolymers is highly complicated, where, thiol-thiol oxidation reactions are carried out to introduce disulfide bonds at the skeleton or the side groups of copolymers. A major setback to this reaction is the readily oxidizing nature of free thiols, even under normal atmospheric conditions, thereby, forming intramolecular disulfide linkages rather than desired intermolecular disulfide linkages (Thambi et al., 2011). Hence, in the present work, a new reaction scheme using cystamine dihydrochloride as disulfide bearing moiety for synthesis of dithiol containing copolymer has been proposed, through which, a novel disulfide-linked chitosan-SS-mPEG copolymer suitable for redox responsive drug delivery has been successfully synthesized. The disulfide containing copolymer was synthesized by grafting cystamine appended chitosan onto carboxylic acid ended mPEG. The synthesized polymeric intermediates and copolymer were characterized in detail, followed by which, polymeric nanoparticles (PNPs) were developed using the synthesized redox responsive copolymer. The formulation characteristics and redox responsive properties were also investigated. Cytotoxicity and cellular uptake studies were carried out for folic acid conjugated PNPs in folic acid binding site overexpressed MCF7 cell lines. Overall, this study afforded an efficient approach for the development of folic acid attached redox responsive polymeric nanoparticles, which has a great potential towards redox responsive site specific drug delivery applications.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation, 85%), cystamine dihydrochloride and methoxy poly(ethylene glycol) (Mw: 2000) were obtained from Sigma Aldrich (Bangalore, India). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Acros Organics (Belgium). Phthalic anhydride and monochloroacetic acid were purchased from Merck (Mumbai, India). Succinic anhydride (SA), dimethyl amino pyridine (DMAP), folic acid (FA) and 5-Fluorouracil (5-FU) were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai. MCF7, human breast adenocarcinoma cell line, was obtained from National Centre for Cell Science (NCCS), (Pune, India). MTT reagent and Minimum Essential Media Eagle (MEM) were purchased from Himedia Laboratories, India. Double distilled water was obtained from Milli-Q Water System. All other commercially available chemicals were used as received.

2.2. Methods

2.2.1. Synthesis of O-carboxymethyl-chitosan

O-Carboxymethyl-chitosan (OCMC) was synthesized according to procedure described by Chen and Park (2003). For the carboxymethyl substitution of chitosan, it was alkalized with 50% aqueous NaOH (20 ml) at − 10 °C for 24 h, followed by which, the alkalized chitosan was allowed to react with 2.5 g monochloroacetic acid at 45 °C for 4 h. The reaction was then arrested by adding 70% ethanol to the reaction mixture, after which, sodium salt of OCMC was separated by filtration. The sodium salt of OCMC was rinsed with 70% ethanol and converted to OCMC by adding HCl. The OCMC thus obtained was filtered and vacuum dried for further use.

2.2.2. Synthesis of O-cystamine-chitosan

Five hundred milligram of OCMC was dissolved in 50 ml of distilled water. EDC (0.4 g) and NHS (0.23 g) were dissolved in distilled water at 25 °C and slowly added to above reaction mixture under nitrogen atmosphere and was stirred at room temperature for 24 h. Thereafter, the solution was purified using a dialysis membrane (MWCO = 1000 Da, Spectrum Laboratories, Inc., CA, USA) against distilled water for 24 h, followed by lyophilization. The yield of the product was 0.4 g (Li et al., 2012).

2.2.3. Synthesis of mPEG-carboxylic acid (mPEG-COOH)

mPEG-COOH was synthesized by dissolving 1.0 g of mPEG in anhydrous dichloromethane under nitrogen atmosphere. Subsequently, SA (0.25 g) and DMAP (0.075 g) were added to mPEG solution, and the whole reaction mixture was kept at room temperature for 48 h under vigorous stirring. The obtained product was then dissolved in dichloromethane and reprecipitated with cold ether. The precipitation procedure was repeated thrice and the resultant precipitate was filtered, dialyzed for 48 h and lyophilized to obtain 0.8 g of desired mPEG-COOH (Casettari et al., 2010).

2.2.4. Synthesis of CH-SS-mPEG and CH-g-mPEG copolymer

Copolymer was synthesized by conjugating the amine group of O-cystamine-chitosan to the carboxylic acid group of mPEG-COOH in the presence of EDC/NHS. EDC initiates the formation of an amide linkage between O-cystamine-chitosan and mPEG-COOH by forming an active intermediate and, NHS increases the stability of the active intermediate. In a typical procedure, O-cystamine-chitosan (0.5 g) and mPEG-COOH (2.3 g) were dissolved in 20 ml of distilled water that contained EDC (0.450 g) and NHS (0.380 g). The reaction was carried out at 25 °C with constant stirring for 24 h. Finally, the solution was dialyzed against deionized water with a dialysis membrane of MWCO 12 kDa (Himedia, India) for 48 h and lyophilized to obtain 0.45 g of desired copolymer. CH-g-mPEG copolymer used as a reduction insensitive control was synthesized by the reaction of chitosan with mPEG-COOH using EDC/NHS as coupling reagent. Finally, the reaction solution was dialyzed and lyophilized to get the copolymer (CH-g-mPEG) (Peng et al., 2010).