Carbohydrate Polymers xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

pH-responsive selenium nanoparticles stabilized by folate-chitosan delivering doxorubicin for overcoming drug-resistant cancer cells

Urarika Luesakul[a], Songchan Puthong[b], Nouri Neamati[c], Nongnuj Muangsin[a,*]

[a] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[b] Antibody Production Research Unit, Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok 10330, Thailand
[c] Department of Medicinal Chemistry, College of Pharmacy, and Translational Oncology Program, University of Michigan, North Campus Research Complex,1600 Huron Parkway, Ann Arbor, MI, 48109, USA

ARTICLE INFO

Keywords:
pH-sensitive
Selenium nanoparticles
Doxorubicin
Chitosan
Multidrug resistance

ABSTRACT

Herein, we first report pH-responsive SeNPs stabilized with modified folic acid-N-trimethyl chitosan (TMC-FA) as nanocarriers for delivery of doxorubicin (DOX) to overcome drug-resistant cancer cells, which could enhance the activity of DOX by approximately 10-fold for a reduced IC_{50} value compared to free DOX. When nanoparticles were taken up by cells, the DOX-loaded SeNPs@TMC-FA demonstrated a faster release rate under acidic conditions. The cumulative release amount of DOX at pH 5.3 was 54.1% within 2 h and 95.5% at 6 h, whereas the release rate at pH 7.4 was 12.3% in 2 h and 42.2% for 6 h; release was not completed at the end of the study, 72 h. Mechanistic studies suggested that DOX-SeNPs@TMC-FA induced cell death through the apoptosis pathway by involvement of caspase-3 and PARP proteins. The results demonstrated that pH-responsive SeNPs@TMC-FA, as targeted nanocarriers, promoted the efficacy of DOX and overcame drug resistance in NCI/ADR-RES cells.

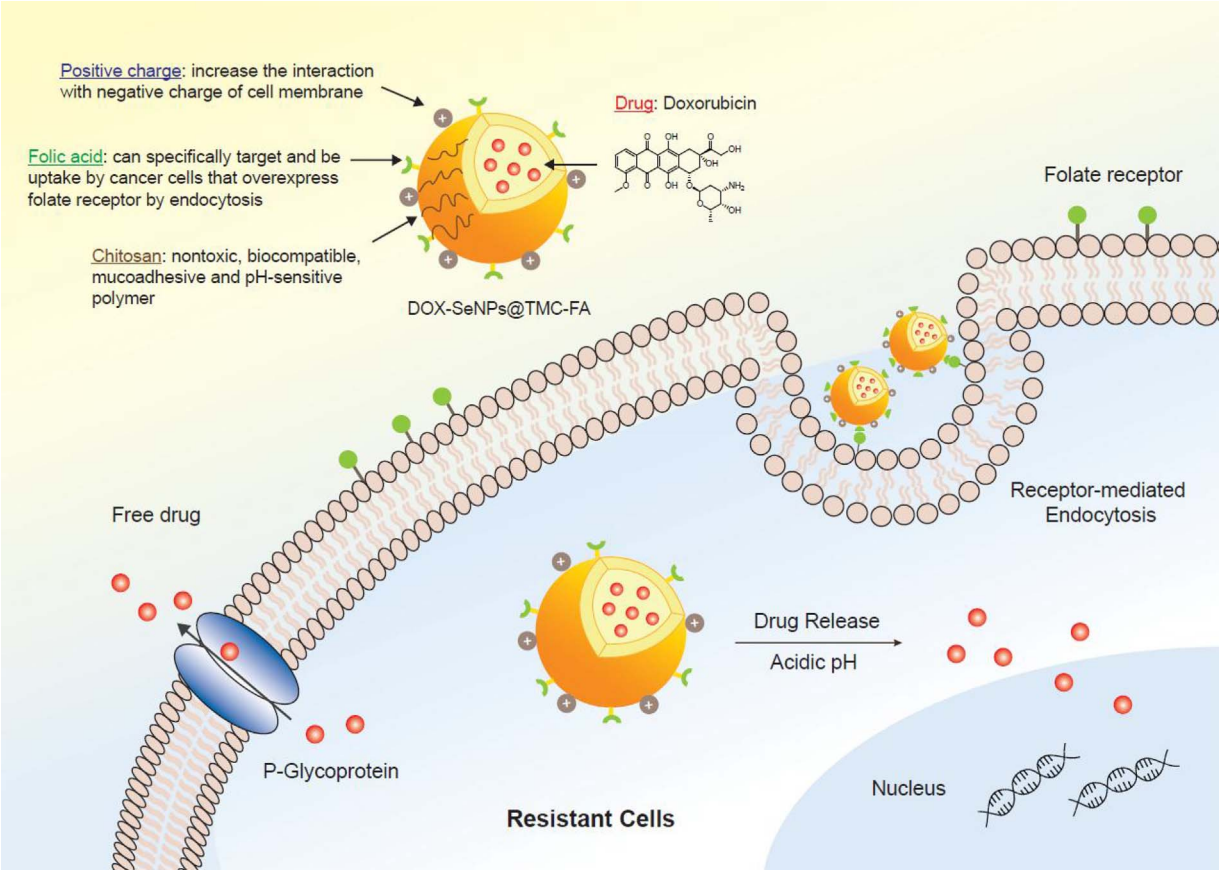
1. Introduction

Currently, doxorubicin (DOX)-based combination chemotherapy is the main therapeutic strategy for various types of carcinomas, but it fails to treat cancers with multidrug resistance or MDR. The most well-documented mechanism of MDR involves drug efflux mediated by ATP-binding cassette (ABC) transporters, typically P-glycoprotein (P-gp) (Wang, Bai, Deng, Fang, & Chen, 2017). P-gp is over-expressed in many human cancers and increases the efflux of intracellular anticancer drugs, leading to low intracellular drug accumulation and down-regulation of the chemotherapy efficacy (Amin, 2013; Zheng et al., 2016). Therefore, overcoming the increased drug efflux is a great challenge for cancer treatments. One of the current strategies for overcoming MDR is using an inhibitor of the drug efflux pump to circumvent the efflux by P-gp. Currently, various small molecules, including natural products, pharmaceutical inert excipients and formulations including cyclosporine A, verapamil and PSC833, have been identified as P-gp inhibitors, but failed in clinical trials due to their poor specificity and high toxicity (Abdallah, Al-Abd, El-Dine, & El-Halawany, 2015). As a potential alternative, nanoparticles such as gold, silica, carbon, and iron oxide, have promising targeted delivery and are relatively nontoxic. These nanoparticles have been designed to overcome the drug resistance of cancer cells by bypassing the P-gp-mediated drug efflux pumps through (i) enhanced biodistribution and cell internalization, (ii) inhibition of drug efflux, (iii) combinational treatments (e.g., siRNA and radiation), or (iv) nanoparticle-mediated endocytosis, as well as increasing intracellular drug accumulation through stimuli-responsive drug release (Li et al., 2015; Liu et al., 2016; Wang, Dou, He, Zhang, & Shen, 2014).

Among those strategies, stimuli-responsive drug release based on pH variation, which is expected to stabilize the drug in the natural environment, has received more attention and rapidly achieves drug release once the pH of the microenvironment reaches the trigger point, resulting in increased intracellular drug accumulation to overcome resistance. Furthermore, in the case of cancer therapy, tumor cells have lower pH values than normal cells because of anaerobic glucose metabolism. The pH difference between normal and cancer cells can also be used for targeted drug delivery to reduce side effects in normal cells; therefore, the pH-triggered approach is one of the most efficient strategies for a drug delivery system. Hence, several groups have worked to develop pH-sensitive nanoparticles to overcome multidrug resistance. For example, a previous study demonstrated chitosan-coated mixed micellar nanocarriers (polyplexes) for co-delivery of siRNA and DOX. The cationic chitosan of was used as the outer coating to allow delivery

* Corresponding author.
E-mail addresses: nongnuj.j@chula.ac.th, nongnuj.ms@gmail.com (N. Muangsin).

https://doi.org/10.1016/j.carbpol.2017.11.068
Received 18 August 2017; Received in revised form 19 October 2017; Accepted 19 November 2017



Scheme 1. the proposed actions of DOX-SeNPs@TMC-FA and pH-triggered intracellular release for overcoming drug resistance in cancer cells.

of siRNA and to provide pH responsiveness. The pH-responsive polyplexes showed enhanced DOX release at acidic, tumor pH values, resulting in higher intracellular accumulation and significantly reduced tumor volume in mice with tumors from a multidrug-resistant cell line (Butt et al., 2016). Rather than using polymer-based NPs, the use of pH-responsive metal nanomaterials loaded with the anticancer drug DOX is another approach to overcome drug resistance. ZnO nanoparticles can bypass P-gp mediated drug efflux, and ZnO dissolves into zinc ions in acidic pH, which achieves the degradation of the nanocarriers once the nanoplatform enters an endosome/lysosome, leading to a rapid, intracellular drug release and therefore, an increase in drug accumulation in resistant breast cells (Liu et al., 2016).

Chitosan (CS) is known for pH-dependent swelling and is a controlled drug release polymer, providing selective drug release in acidic intracellular vesicles such as endosomes and lysosomes in targeted tumor cells (Unsoy, Khodadust, Yalcin, Mutlu, & Gunduz, 2014). It has been reported that CS provides pH-responsive drug release because its amino groups are protonated at acidic pH. Importantly, various studies have shown that chitosan-based nanocarriers can overcome MDR in cancer cells. For example, chitosan coated magnetic nanoparticles have been synthesized as a pH-responsive drug delivery system, which is targeted to tumor cells under a magnetic field; this is an efficient system to eliminate DOX resistance in MCF-7/Dox cells (Unsoy et al., 2014). Furthermore, chitosan-coated mixed micellar nanocarriers were prepared for co-delivery of siRNA and DOX. These particles showed pH-responsive behaviors with enhanced DOX release at acidic, tumor pH values, resulting in higher intracellular accumulation and enhanced cytotoxicity in drug-resistant cells (Butt et al., 2016).

Selenium nanoparticles (SeNPs) have received considerable attention as both potential cancer therapeutic agents and drug carriers due to their biocompatibility, low toxicity, and excellent chemopreventative effects (Saraswathy and Gong, 2014; Zheng et al., 2015). To our knowledge, there are few previous reports on the design and development of SeNPs to overcome MDR. For instance, the first report demonstrated a nanoparticle-mediated endocytosis strategy using folate (FA)-conjugated SeNPs as a cancer-targeted nanodrug delivery system, which can bypass the P-gp-mediated drug efflux pumps through FA receptor-mediated endocytosis to overcome the MDR in R-HepG2 cells (Liu et al., 2015). Other studies have designed combinational treatments of genes (siRNA) and/or drugs, including a Ru(II) complex and cisplatin using SeNPs as nanocarriers, which achieved efficacy in vitro and overcame MDR (Chen et al., 2015; Zheng et al., 2015; Zheng et al., 2016).

Taken together, in this study, to overcome drug resistance effectively, we have designed a selenium nanodrug delivery system by combining pH-responsive drug release for increased intracellular accumulation in specific positions with FA-mediated endocytosis for bypassing the P-gp-mediated drug efflux pumps to overcome MDR. Chitosan is also widely used as a stabilizer for various nanoparticles because of its nontoxicity, high biodegradability and good biocompatibility (Luesakul, Komenek, Puthong, & Muangsin, 2016). Since chitosan possesses a pKa value of 6.5, its water solubility under physiological conditions is significantly limited (Fowler et al., 2014). In our previous study, we reported chitosan modified to have a positive charge; N-trimethyl chitosan (TMC) to improve their solubility. Furthermore, folic acid and its conjugates have been well investigated in recent years to specifically target the folate receptor which is overexpressed in human tumor cells. We, therefore, modified folic acid-N-trimethyl chitosan (TMC-FA) and folic acid-gallic acid-N-trimethyl chitosan (FA-GA-TMC) and used these compounds as stabilizers for the fabrication of SeNPs (Luesakul et al., 2016). As mentioned before, in this work, pH-responsive NPs are combined with targeted nanocarriers in order to control drug release and increase drug intracellular accumulation; therefore, TMC-FA was selected as a stabilizer for the