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Selective redox-responsive drug release in tumor cells mediated by chitosan based glycolipid-like nanocarrier

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ABSTRACT

The redox responsive nanocarriers have made a considerable progress in achieving triggered drug release by responding to the endogenous occurring difference between the extra- and intra- cellular redox environments. Despite the promises, this redox difference exists both in normal and tumor tissue. So a non-selective redox responsive drug delivery system may result in an undesired drug release in normal cells and relevant side-effects. To overcome these limitations, we have developed a chitosan based glycolipid-like nanocarrier (CSO-ss-SA) which selectively responded to the reducing environment in tumor cells. The CSO-ss-SA showed an improved reduction-sensitivity which only fast degraded and released drug in 10 mM levels of glutathione (GSH). The CSO-ss-SA could transport the drug fast into the human ovarian cancer SKOV-3 cells and human normal liver L-02 cells by internalization, but only fast release drug in SKOV-3 cells. By regulating the intracellular GSH concentration in SKOV-3 cells, it indicated that the cellular inhibition of the PTX-loaded CSO-ss-SA showed a positive correlation with the GSH concentration. The CSO-ss-SA was mainly located in the liver, spleen and tumor in vivo, which evidenced the passive tumor targeting ability. Despite the high uptake of liver and spleen, drug release was mainly occurred in tumor. PTX-loaded CSO-ss-SA achieved a remarkable tumor growth inhibition effect with rather low dose of PTX. This study demonstrates that a smartly designed glycolipid-like nanocarrier with selective redox sensitivity could serve as an excellent platform to achieve minimal toxicity and rapid intracellular drug release in tumor cells.

1. Introduction

During the past few decades, much effort has been concentrated on stimuli-responsive nanocarriers to maximize the therapeutic potential of drug molecules at target tissue and minimize their effects in healthy ones [1–5]. Especially, as one of the most studied stimuli-responsive systems, the redox-responsive nanocarriers show a superior ability and have made a considerable progress in achieving triggered drug release by responding to the endogenous occurring difference between the extra- and intra-cellular redox environments [6–10].

It should be noted that although the in vitro proofs of concept have been reported for a number of redox-responsive systems, only a few have been tested comprehensively and proven to be effective in in vivo models [11,12]. Many studies were more interested in the sensitive ability of the drug release mediated by the redox-responsive nanocarrier, but ignored the selective ability of the sites where the redox reactions took place [13,14]. In spite of the low reducing potential, the redox reactions between the redox-responsive systems and surroundings can still take place during circulation in the blood and in the extra-cellular space [15–18]. Even more, the difference between extra- and intra-cellular redox environments is ubiquitous in vivo [19,20], which means that the normal and tumor tissues both have this redox potential difference. So a non-selective redox responsive drug delivery system may result in premature drug release before reaching the tumor cells, undesired drug release in normal cells and relevant side-effects and toxicity.

However, there is an elevated production of reducing substances (7–10 folds) in tumor cells than normal cells in spite of the extra- and intra-cellular redox difference [21,22]. With a more rational design, a selective redox-responsive drug delivery system (DDS) with the cleavage rate is minimal in circulation and normal cells, while proving sufficiently fast in the tumor cells may serve as a feasible solution.

According to these principles and our recent research basis, we proposed a chitosan-based glycolipid-like redox-responsive nanocarrier. Chitosan with positive zeta potential is biodegradable and low immunogenicity. Lipid is compatible with the cellular membrane, and is confirmed to promote the cellular uptake of the encapsulated drug. And the main advantages of chitosan-based glycolipid-like nanoparticle for DDS are rapid intracellular uptake capacity, suitable drug loading capacity, and easy surface modification ability. So the chitosan-g-stearic acid (CSO-SA) has been exploited for potential DDS by our research

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group and gained a certain success [23–25]. But with amide bonds as the linkers, which have a slow degradation rate within the cell, the drug release from the CSO-SA is relatively slow. Therefore, reducible disulfide bonds linked glycolipid-like nanocarrier may provide an option for drug delivery.

In this study, we have developed a chitosan based glycolipid-like nanocarrier (CSO-ss-SA) to selectively respond to the reducing environment in tumor cells. Hydrophobic antitumor drug paclitaxel (PTX) and Nile red fluorescence marker were used as the model drugs. We studied the stability and reduction sensitivity in different reduction environments simulated with the blood plasma, normal cells and tumor cells. And we investigated the intracellular redox sensitivity of CSO-ss-SA against human ovarian cancer SKOV-3 cells, human normal liver L-02 cells and human ovarian surface epithelial HOSEpiC cells. Then we discussed the relationship between the reduction sensitivity and cytotoxicity. The tumor xenograft model on BALB/C+/nu mice was also used to investigate the distribution, the in vivo drug release and antitumor activity. With the selective redox sensitivity of CSO-ss-SA, we demonstrated the possibility of utilizing naturally occurring redox changes to achieve minimal toxicity and rapid intracellular drug release in tumor cells (Fig. 1A).

2. Materials and methods

2.1. Materials

Chitosan with an approximate 15.0 kDa average molecular weight was obtained by enzymatic degradation of 95% deacetylated chitosan (Mw = 450 kDa) and was supplied by Yuhuan Marine (Yuhuan, China). Octadecylamine was purchased from Fluka (Milwaukee, WI, USA). The 3, 3′-dithiodipropionic acid was purchased from Tokyo Chemical Industry (Tokyo, Japan). Paclitaxel (PTX) was purchased from Shanghai Zhongxi Sunve (Shanghai, China). Taxol was purchased from Bristol-Myers Squibb (New York, USA). L-Glutathione (GSH) and Nile red (NR) were purchased from Sigma-Aldrich (Diegem, Belgium). Fluorescein isothiocyanate (FITC), 2,4,6-trinitrobenzene sulfonic acid

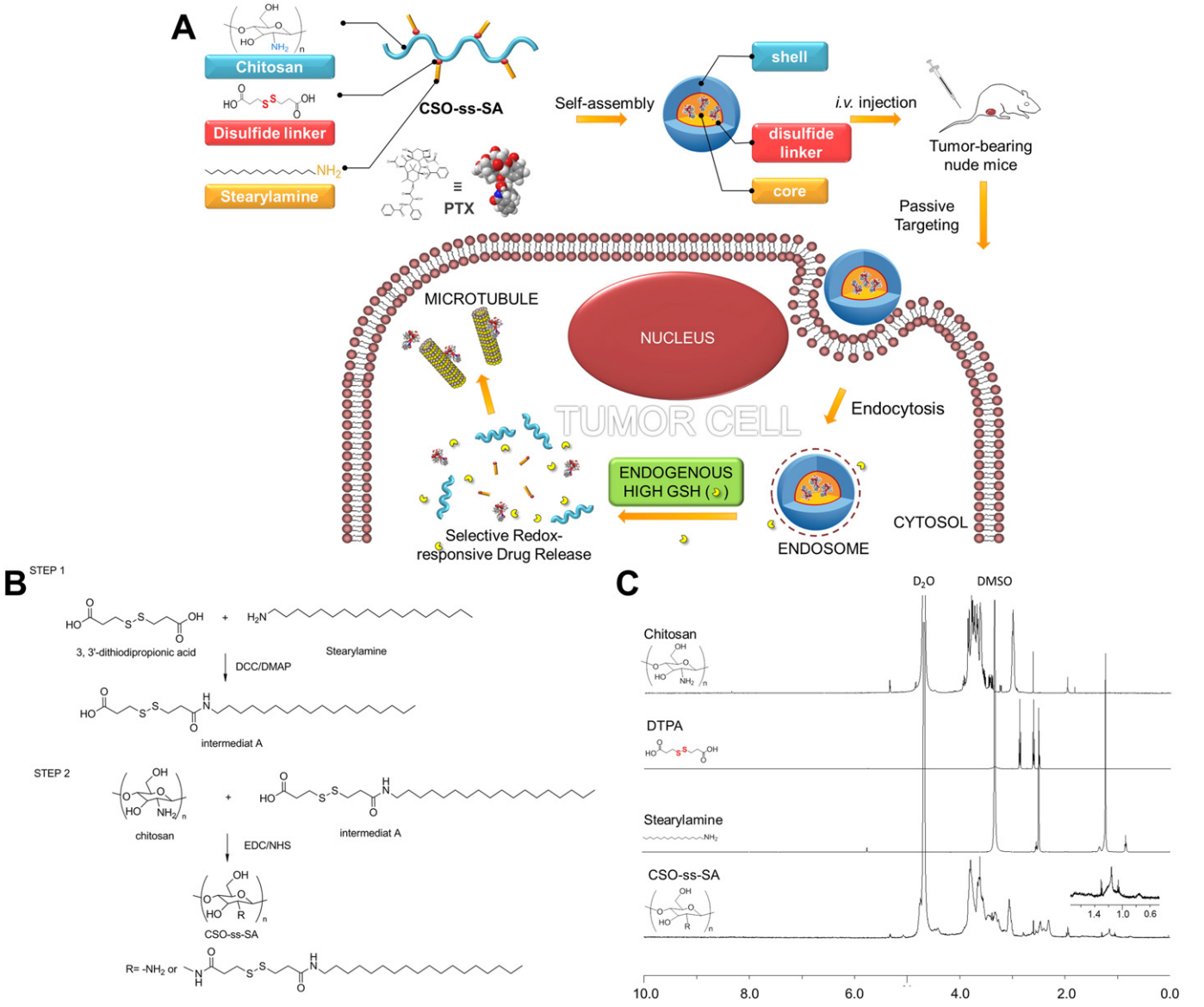

Fig. 1. The schematic diagram of CSO-ss-SA. (A) Schematic structure of CSO-ss-SA, self-assembly into micelles, passive target into tumor tissue and response to the endogenous high GSH in tumor cells. (B) Synthesis route of CSO-ss-SA via the disulfide linkers. (C) ^{1}H NMR spectra, from top to bottom: chitosan, DTPA, ODA, and CSO-ss-SA. The signals assigned to the -CH_2- (1.2 ppm) ODA were detectable on the CSO-ss-SA spectra.