MUC 1 aptamer conjugated to chitosan nanoparticles, an efficient targeted carrier designed for SN38 delivery toward colon cancer

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ABSTRACT SUMMARY

In this study we attempted to combine the technology of controlled release drug delivery and targeted drug delivery by surface engineering of polymeric nanoparticles with targeting aptamer. We specifically designed chitosan nanoparticles as carrier for SN-38 and conjugate MUC1 aptamer to the surface of chitosan nanoparticles in order to target nanoparticles for colon cancer cells.

INTRODUCTION

Irinotecan (CPT-11) is a semisynthetic derivative of camptothecin exhibiting proper water solubility and hindering cell nucleic acid synthesis by inhibiting topoisomerase I enzyme function. In spite of good solubility, only 5-10% of the administrated dose will be converted to the SN-38 (7-ethyl 10-hydroxy Camptothecin) which is the active form of the drug and has hydrophobic properties; besides this conversion shows unpredictable inter-patient varieties. Conventional chemotherapeutic agents affect cancerous cell as well as normal cells and cause undesirable side effects. Developing nanoparticles (NPs) of drug-polymer conjugates can be a good candidate for overcoming such problems. Furthermore, active targeting is a solution for lowering cytotoxicity effect and increasing efficacy of carries. Among different targeting agents, aptamer has a better specificity and low immunogenic properties that candidate them as an excellent targeting moieties. At this study, chitosan was used as a nanocarrier for delivery of SN38 to colon cancer. For this purpose, N-carboxyethyl chitosan ethyl ester (CS-EA) was synthesized as follows: 250 mg of prepared chitosan was dissolved in 2% (v/v) acetic acid and then diluted with 50 ml ethanol. 0.5 ml Ethyl acrylate was then added to the solution. The product was dialyzed and lyophilized to obtain CS-EA. To convert the ester of the prepared product to carboxyl group 150 mg of lyophilized CS-EA was dissolved in 4.5 ml acetic acid (5% v/v). The reaction was ended up by adding NaOH (1 M) to precipitate CS-AC. Conjugation of SN38 to chitosan was achieved by dissolving 100mg of CS-AC in 5ml of PBS. The reaction followed by addition of 200mg EDC and 105 mg NHS. 350 mg BOC-SN38 was dissolved in 35 ml DMSO and the aforementioned aqueous part was added dropwise into it and the reaction continued for 24 hours. The solution was dialyzed against methanol and deionized water and lyophilized to obtain yellowish powder. Conjugation of CS-SN38 was confirmed by differential scanning calorimetry (DSC), fourier transform infrared (FTIR) spectroscopy, and ¹H Nuclear Magnetic Resonance (H NMR) spectroscopy. For preparation of NPs Nanoparticles ionotropic gelation method was used. Briefly, The CS-SN38 conjugate was dissolved in deionized water and acetic acid was dropped to the solution. The solution was treated with ultrasonic radiation and tripolyposphate (TPP) was also added during the process. Size and morphology of the prepared NPs were analyzed using dynamic light scattering (DLS) and scanning electron microscopy (SEM). Aptamer modified nanoparticles were prepared through reaction with EDC and NHS which was confirmed by agarose gel electrophoresis. In vitro cytotoxicity of the prepared NPs were assessed in cell culture using HT-29 cell line as MUC 1 positive cell through MTT assay and Cellular uptake was also confirmed via confocal imaging.

EXPERIMENTAL METHODS

H NMR and FTIR spectroscopic study of CS-SN38 is illustrated in figure 1 and 2. Besides chitosan protons, methyl, ethyl and aromatic protons of SN38 can be seen
respectively at 0.942 ppm to 1.154 ppm and at 2.98 ppm to 3.095 ppm and at 7.097 ppm to 8.33 ppm. Ester bond between CS-AC and SN38 can be detected from FTIR spectrum peak around 1756.58 cm⁻¹.

Ethyl acrylate grafting to CS-EA was 36% and the amount of drug (SN38) conjugated to chitosan (CS-SN38) was found to be %7.09±0.12 via spectrophotometry. Prepared nanoparticles size was around 176 nm and they were spherical and had smooth surface. Conjugation of aptamer to nanoparticles was confirmed by gel electrophoresis retardation assay which is shown in figure 3.

Cell viability evaluation of NPs via MTT assay confirms that CS-SN38–Apt nanoparticles have equal cytotoxicity in comparison with SN38 and increased toxicity in comparison with CS-SN38 nanoparticles. The cellular uptake of the NPs was confirmed by confocal microscopy. HT-29 and CHO cells were seeded in six-well cell culture plate and then incubated by NPs and aptamer targeted NPs (Apt-NP) and then washed with DMEM and PBS to remove the NPs and NPs-Apt that did not enter the cells. The cells were then stained with DAPI solution before being monitored using the confocal microscope. Figure 4 demonstrate NPs-Apt uptake on HT-29 cell line.

CONCLUSION

In this study a novel targeted drug delivery system based on chitosan has been developed. DNA based aptamer has been used as a targeting agent. The final conjugate has drug content of %7.09±0.12 (w/w) and nanoparticle prepared via ionotropic gelation leading to nanoparticle ranging from 100-300 nm. The final size depended on many factor such as polymer concentration. The targeted delivery system had the same toxicity respect to free drug on HT29 cell line. However; in vivo studies can evaluate the efficacy of our prepared system more precisely.

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