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Cationic core-shell nanoparticles for intravesical chemotherapy in tumor-induced rat model: Safety and efficacy

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ABSTRACT

Mitomycin C (MMC) has shown potent efficacy against a wide spectrum of cancers and is clinical first choice in superficial bladder tumors. However, intravesical chemotherapy with MMC has been ineffective due to periodical discharge of the bladder and instability of this drug in acidic pH, both resulting in high rate of tumor recurrence and insufficiency to prevent progression. Nanocarriers may be a promising alternative for prolonged, effective and safe intravesical drug delivery due to their favorable size, surface properties and optimum interaction with mucosal layer of the bladder wall. Hence, the aim of this study was to evaluate and optimize cationic core-shell nanoparticles formulations (based on chitosan (CS) and poly-ε-caprolactone (PCL)) in terms of antitumor efficacy after intravesical administration in bladder tumor induced rat model. Antitumor efficacy was determined through the parameters of survival rate and nanoparticle penetration into the bladder tissue. Safety of the formulations were evaluated by histopathological evaluation of bladder tissue as well as observation of animals treated with MMC bound to nanoparticles. Results indicated that chitosan coated poly-ε-caprolactone (CS-PCL) nanoparticles presented the longest survival rate among all treatment groups as evaluated by Kaplan–Meier plotting. Histopathological evaluation revealed that cationic nanoparticles were localized and accumulated in the bladder tissue. As intravesical chemotherapy is a local therapy, no MMC was quantified in blood after intravesical instillation indicating no systemic uptake for the drug which could have subsequently led to side effects. In conclusion, core-shell type cationic nanoparticles may be effective tools for the intravesical chemotherapy of recurrent bladder tumors.

1. Introduction

Bladder cancer is one of the most common cancers worldwide, accounting for 3.2% of all cancers (Ferlay et al., 2001). 70% of bladder cancers are non-muscle invasive and are managed by an endoscopic resection procedure, however 50–70% of non-muscle invasive tumors come back within five years after transurethral resection (TUR) and 20–30% of recurrent disease progresses to higher stage or grade resulting in metastasis of the tumor (Miyake et al., 2004; Lehmann et al., 2006). The most common therapeutic approach in bladder cancer is intravesical therapy in which drugs are directly administered into the bladder to reduce or prevent tumor recurrence progression through local chemotherapy (Malmstrom, 2003). The most commonly employed intravesical agents in patients with superficial bladder cancer are mitomycin C (MMC), thiotepa, ethoglucid, anthracyclines such as doxorubicin and epirubicin, Bacille Calmette Guerin, taxol and the new mitomycin derivative KW-2149 (Heijden van der and Witjes, 2003). Intravesical drug delivery may be ineffective due to several factors mostly arising from bladder physiology. The most important drawback is the periodical need of discharge of the bladder approximately every 2 h. Therefore, even the drug is administered locally to the therapeutic site, it is reported to be rapidly diluted and lost resulting in repeated catheterization of the patient and ineffective chemotherapy (Tyagi et al., 2004; Parekh et al., 2006; Kaufman, 2006). Another disadvantage is the very low permeability of urothelium which is called the bladder permeability barrier (BPB) (Tyagi et al., 2006). Urothelium, is composed of

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glycosaminoglycan (GAG) mucin layer and this prevents the adhesion of particles to bladder mucosa and limits the mucosal absorption of molecules into deeper tissues (Tyagi et al., 2004; Parekh et al., 2006). Several approaches to improve intravesical drug delivery has been developed aiming to enhance the permeability of drugs through the bladder wall. Physical approaches include electromotive drug administration and iontophoresis/electroporation for drug delivery; chemical approaches include administration in solvents such as DMSO. Finally technological approaches mainly focus on increasing the residence time of dosage forms in the bladder via bioadhesive colloidal carriers and nanotechnology for improved permeability of drugs and prolonged residence of drug delivery systems in the bladder (Giannantoni et al., 2006). In our previous studies cationic nanoparticles were developed by either coating PCL nanoparticles with CS or PLL or directly from CS nanoparticles for the prolonged residence of MMC in rat bladder (Erdogar et al., 2012) and improved cellular uptake by MB49 mouse bladder carcinoma cell line (Bilensoy et al., 2009). Cationic nanoparticles for effective MMC delivery were optimized for higher drug loading capacity and smaller particle size by means of different preparation techniques in our previous studies (Bilensoy et al., 2009; Erdogar et al., 2012) and CS, CS-PCL and PLL-PCL nanoparticles were evaluated for cellular uptake and cytotoxicity properties. In the light of these studies, PLL-PCL nanoparticles were not included in animal studies as they were found to be highly toxic in cell culture studies. Formulations with highest drug loading values and smallest size were used in the in vivo studies.

Mitomycin C (MMC) is an antitumor antibiotic exerting therapeutic activity against many human neoplasms (Carter et al., 1979) and clinical choice in intravesical chemotherapy of superficial bladder tumors. The MMC is known to rapidly degrade in acidic environment (Stolk et al., 1986) and cause allergic reactions that are dose-dependent upon systemic uptake and also chemical cystitis (Thrasher and Crawford, 1992).

Chitosan nanoparticles have shown mucoadhesive properties from the semi-synthetic cationic polymer chitosan that has a well-known bioadhesive nature, by the establishment of electrostatic interactions with sialic groups of mucins in the mucus layer. As a result of this, chitosan promote a structural reorganization of the tight junction-associated proteins and enhance the absorption of hydrophilic drugs (Bravo-Osuna et al., 2007).

To overcome these problems and to prolong the residence of the chemotherapeutic drug in the bladder, cationic core shell nanoparticles of PCL coated with cationic polymers CS was evaluated in comparison to MMC commercial product in solution form in bladder tumor induced rat model as a follow-up study of our previous work (Erdogar et al., 2012) comprising in vitro, ex vivo and in vivo evaluation of intravesical chemotherapy with cationic nanoparticles.

2. Materials and methods

2.1. Materials

The following materials were purchased from various companies and then used as received. Chitosan (Protasan UPLC 113 viscosity <20 mPa/s $M_w < 200$ (kDa)^2) was obtained from FMC Biopolymers, Norway. Active ingredient (mitomycin C) was a kind gift from the pharmaceutical company Onko-Koçsel, Turkey. Pluronic® F-68, polyvinylalcohol (PVA), sodium tripolyphosphate (Na TPP) were obtained from Sigma–Aldrich. Poly-ε-caprolactone (PCL) (Mn: 42.500) was purchased from Aldrich (St. Louis, MO, United States). Methylene chloride was obtained from J. T. Baker, United States. Ethyl acetate, chloroform, methanol and 2-propanol, *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) were purchased from Sigma–Aldrich (St. Louis, MO, United States). All other chemicals were of reagent grade and solvents were of HPLC grade.

2.2. Preparation and characterization of nanoparticles

2.2.1. Preparation of nanoparticles

2.2.1.1. Core-shell nanoparticles. The MMC-CS-PCL nanoparticles were prepared by the W/O/W double emulsion technique. The adjustment of the technique was based on the use of a homogenizer thus reducing considerably the size of droplets. Briefly, 2 mL 1% PF68 (w/v) solution containing 5 mg MMC (10% of PCL weight) was emulsified in 10 mL methylene chloride containing 0.5% PCL by ultraturrax (IKA T25 basic, Germany) at 16,000 rpm for 5 min over an ice bath to form the innerphase. This primary emulsion was immediately injected into 40 mL of aqueous solution containing 0.1% (w/v) PVA and 0.05% CS in 1% PF68 (w/v) solution for MMC-CS-PCL nanoparticles, using a glass syringe with a needle under agitation. Then the double emulsion was stirred by Ultraturrax high speed homogenizer at 16,000 rpm for 5 min over an ice bath. Finally, nanoparticles were obtained in final form after removal of organic solvent under vacuum (IKA-Werke-RV06 ML, Germany) at 37 °C to the desired volume (42 mL) (Hasan et al., 2007; Lamprecht et al., 1999).

2.2.1.2. Core nanoparticles. The MMC-CS nanoparticles were prepared by ionotropic gelation technique based on interaction of oppositely charged groups of chitosan and sodium tripolyphosphate to form nanoparticles spontaneously. The MMC was dissolved in TPP solution (20% of polymer weight) and then the TPP solution (0.4 mg/mL) was added to the CS aqueous solution (1.75 mg/mL) and stirred at room temperature. Spontaneously formed nanoparticles were further separated by centrifugation at 13,500 rpm for 1 h and discarding of the supernatant and redispersion of the precipitate in water.

2.2.2. Characterization of nanoparticles

Particle size and size distribution, zeta potential, surface morphology, encapsulation efficiency and in vitro release studies were preformed within the scope of in vitro characterization studies as follows:

Mean diameter (nm) and polydispersity index values of blank and drug loaded nanoparticles were determined by quasi-elastic light scattering technique (QELS) using Malvern NanoZS (Zetasizer NanoSeries ZS, Malvern Instruments, UK). Analyses were performed in triplicate at 25 °C at a 90° angle.

Zeta potential of nanoparticle dispersions were determined to confirm the surface charge of the particles using Malvern NanoZS (Zetasizer NanoSeries ZS, Malvern Instruments, UK) in triplicate at 120° angle and 25 °C.

For the determination of drug loading, nanoparticles were separated from the aqueous suspension by ultracentrifugation at 13,500 rpm for 1 h. The amount of free MMC in the supernatant was directly analyzed by an analytically validated HPLC technique ($r^2 = 0.9995$) The HPLC method for the quantification of MMC consisted of an HP Agilent 1100 HPLC system with a reverse phase C18 column (150 mm × 4.6 mm, Nucleosil 5C18), a mobile phase of acetonitrile:water (15:85 v/v), injection volume – 50 μL and flow rate – 1.5 mL/dk. The DAD detector was set at 365 nm. The percentage drug loading was calculated according to the following equation:

$$\%\text{Drugloading} = \frac{\text{InitialMMC} - \text{FreeMMC}}{\text{InitialMMC}} \times 100$$

In our previous study, pH 6 and pH 7.8 buffers were selected as release media since urine pH is 6 and the MMC is reported to be