Biomaterials 34 (2013) 10258–10266

Contents lists available at ScienceDirect

Biomaterials

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

A magnetic chitosan hydrogel for sustained and prolonged delivery of Bacillus Calmette–Guérin in the treatment of bladder cancer

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ARTICLE INFO

Article history:
Received 4 August 2013
Accepted 6 September 2013
Available online 24 September 2013

Keywords:
Bacillus Calmette–Guérin
Intravesical instillation
Chitosan
Magnetic nanoparticle
Thermosensitive hydrogel
Intravesical drug delivery

ABSTRACT

The aim of this study was to develop a magnetic thermosensitive hydrogel as intravesical Bacillus Calmette–Guérin (BCG) delivery system, which was formulated with chitosan (CS), β-glycerophosphate (GP) and Fe_3O_4 magnetic nanoparticle (Fe_3O_4-MNP). The gelation time and magnetic response of the gel system were investigated. The morphology of the gel was displayed by scanning electron microscope. Frozen section examination was creatively employed for exhibiting the structure of the gel and determining its intravesical residence time. The antitumor effect and local immune activity of BCG loaded magnetic gel were evaluated. The flowing solution of CS/GP under room temperature could gelate rapidly at body temperature both in vitro and in vivo. The magnetic injectable hydrogels significantly prolonged intravesical BCG residence time under an applied magnetic field. In comparison to traditional BCG therapy for superficial bladder tumor, BCG delivered by the gel system induced a stronger Th1 immune response and revealed higher antitumor efficacy.

1. Introduction

In 1976, Bacillus Calmette–Guérin (BCG) was initially proposed to treat superficial bladder cancer by Morales and colleagues [1]. Nowadays intravesical BCG instillations have proven to be the most successful adjuvant treatment for patients with non-muscle-invasive bladder cancer (NMIBC) [2]. However, 30–50% of patients will fail initial BCG therapy [3,4]. Some patients receiving intravesical BCG therapy may suffer severe adverse effects, leading to cessation of BCG therapy [5]. To improve its antitumor effects and/or reduce the side effects, interesting approaches have been developed, such as genetically engineered BCG secreting relevant cytokines, administering various inflammatory cytokines and chemokines in addition to intravesical BCG and instillation of mycobacterial cell wall or its extracts as an alternative to BCG, etc [5–7].

Responses to intravesical therapy are directly proportional to drug concentration rather than drug dose [8]. The duration of BCG exposure is crucial for therapeutic response. However, the BCG exposure at the urothelium rarely lasts beyond the first voiding of urine after instillation, and often patients do not completely respond or the response is highly variable among patients [9]. Strategies such as complete bladder emptying just before dose administration and restricted fluid intake have limited value. Inadequate conventional BCG delivery justifies the search for new vehicles to overcome the limitations inherent in intravesical route of BCG administration.

Thermosensitive hydrogel based on chitosan (CS) and β-glycerophosphate (GP) is currently a promising candidate. It has great potential in various applications, such as drug delivery, cell encapsulation and tissue engineering [10–13]. Aqueous solutions of CS/GP form a free-flowing solution at room temperature and become a viscous hydrogel at body temperature [11]. Upon incorporation of pharmaceutical agents, the hydrogel system could act as a sustained drug release depot in situ [10,12]. The therapeutic benefit of this system was demonstrated by delivering an anti-inflammatory drug in an interstitial cystitis rat model [8]. In the light of the interesting application, we proposed that this polymer could extend the residence time of BCG in bladder. Thereby increase in efficacy of BCG would be expected.

Thermosensitive hydrogels form in bladder could still get washed out of the bladder during voiding, necessitating creative

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http://dx.doi.org/10.1016/j.biomaterials.2013.09.027

methods to make them resistant to excretion. An interesting study by Leakakos et al. attracted our attentions [14]. Prolonged retention and targeting of doxorubin in swine bladder were achieved by instilling magnetic targeted carriers (MTC) composed of metallic iron and doxorubicin adsorbed onto activated carbon via an externally applied magnetic field to the bladder. Inspired by the experiment, we considered that magnetic particles could be utilized to prevent the thermosensitive hydrogels from being washed away during urine voiding. Fe_3O_4 magnetic nanoparticle (Fe_3O_4-MNP) is a kind of magnetic ferric oxide and exhibits good superparamagnetic behavior [15]. The nanoparticles are in preclinical studies as promising drug delivery formulations [16]. When incorporated into thermosensitive hydrogel, they could respond to an external magnetic field and ensure attachment to the bladder wall [16].

Thus, considering the features of formulations, an in situ gel system that contained Fe_3O_4-MNPs and BCG dispersed within the thermosensitive hydrogel based on CS and GP for BCG instillation was developed (Fig. 1a). It was expected that the gel system would extend the duration of BCG in bladder and increase the antitumor efficacy. Motivated by these considerations, the present study was to investigate the feasibility of the gel system, characterize its bladder retention and evaluate the local immune activity.

2. Materials and methods

2.1. Materials

Chitosan powder (95% deacetylated, $M_W = 50{,}000$) was purchased from Jinan Haidebei Marine Bioeeing Co. Ltd. (Shandong, China). Analytical grade β-glycerophosphate was acquired from Sigma–Aldrich (St. Louis, USA). Fe_3O_4-MNP was obtained from Changsha Jingkang Co., Ltd. (Hubei, China). The lyophilized BCG, Pasteur substrain, was supplied by Beijing Institute of Biological Products (Beijing, China). N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN) was also purchased from Sigma–Aldrich. The following reagents were purchased from the suppliers indicated: antibody to CD4 from Abcam (Hong Kong, China); ELISA kits for interleukin-2 (IL-2) and interferon-γ (IFN-γ) from CoWin Biotech (Beijing, China).

Female Wistar rats, 8-week old and about 250 g weight, were housed under barrier environment in our Experimental Animal Center. All rats were maintained in an air-conditioned room and subjected to 12-h dark/light cycles. Animals were handled in accordance with the guidelines of Animal Care Council.

2.2. Preparation of CS and GP solution (CS/GP) and CS/GP solution loaded with Fe_3O_4-MNP and BCG (Fe_3O_4–BCG–CS/GP)

The CS/GP solution was prepared essentially as described before [17]. Briefly, the CS powder was dissolved in 0.1 M hydrochloric acid under stirring for 2 h at room temperature. The insoluble particles in the chitosan solution were removed by filtration. The GP solution was prepared by dissolving the GP powder in distilled water. The two solutions were chilled at 4 °C for 10 min, followed by addition of the GP solution to the CS solution dropwise under stirring at 4 °C until a clear and homogeneous CS/GP solution was formed.

The mixture for instillation was prepared by adding Fe_3O_4-MNPs and BCG powder to the CS/GP solution under stirring and then dispersing with ultrasound.

2.3. Gelation time of CS and GP solution and Fe_3O_4–BCG–CS/GP mixture

The gelation time of CS/GP solution at 37 °C was determined by a typical method [17]. The CS/GP solution was added to a centrifuge tube and heated in a water bath (37 °C). At fixed time intervals, the tube was taken out and inverted. The sample that had not flowed for 30 s in the inverted tube was considered to form a hydrogel, and the time point was recorded as the gelation time. The effect of CS and GP concentration on gelation time was investigated.

The resultant Fe_3O_4–BCG–CS/GP mixture was also incubated at 37 °C, and the gelation time was measured.

2.4. In vivo gelation and frozen section examination

The gelation in vivo was investigated in 3 female Wistar rats. Before instillation, each rat was anesthetized with 3% pentobarbital (30 mg/kg) through intraperitoneal injection and a shortened 3F epidural anesthesia catheter was inserted through the urethra to the bladder lumen. The remaining urine was aspirated with a 1 cc syringe, and then 0.1 ml Fe_3O_4–BCG–CS/GP mixture containing 1 mg of BCG was instilled via the catheter. The time to sacrifice depended on the gelation time determined before in vitro. The bladder neck was ligated and the whole bladder was removed for cryosection. The 10 μm thick sections were stained with hematoxylin-eosin staining (HE staining).

2.5. Scanning electron microscopy (SEM)

SEM was employed to display the surface morphology of the Fe_3O_4–BCG–CS/GP gel. The freeze-dried samples were fixed on adhesive carbon tapes, and then gold coated with a Hitachi HUS-5GB sputter coater (Tokyo, Japan). The SEM images were obtained by using a Hitachi H-8010 scanning system (an attachment of H-800 transmission electron microscope) with the acceleration voltage of 75–100 kV.

2.6. In vivo evaluation of retention

The retention of the Fe_3O_4–BCG–CS/GP gel in bladder was determined in 36 female rats. Water intake of rats was banned 2 h before and after instillation. The

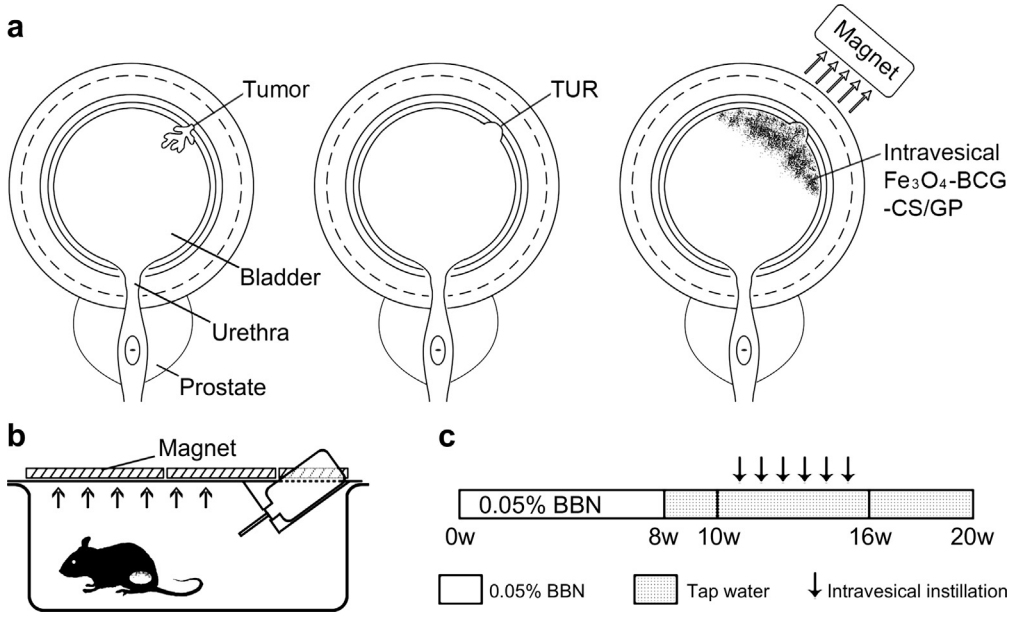

Fig. 1. (a) Schematic illustration of clinical application of the delivery system. (b) A diagram of cage for rats receiving Fe_3O_4–BCG–CS/GP mixture. The Fe_3O_4–BCG–CS/GP gel formed in bladder can be attracted and attached to the bladder wall in the magnetic field generated by the magnets on the cage. (c) Treatment protocol. TUR: transurethral resection.