In Situ Floating Hydrogel for Intravesical Delivery of Adriamycin Without Blocking Urinary Tract

TINGSHENG LIN,[1,2,3] JINHUI WU,[1,4] XIAOZHI ZHAO,[2] HUIBO LIAN,[2] AHU YUAN,[1] XIAOLEI TANG,[1] SAI ZHAO,[1] HONGQIAN GUO,[2] YIQIAO HU[1,4]

[1]State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, China
[2]The Department of Urology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing 210093, China
[3]Medical School of Nanjing University, Nanjing 210093, China
[4]Jiangsu Key Laboratory for Nano Technology, Nanjing 210093, China

Received 29 May 2013; revised 19 December 2013; accepted 23 December 2013

Published online 21 January 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23854

ABSTRACT: Drug solution is commonly used in conventional intravesical instillation. However, most of them would be easily eliminated by voiding, which significantly limit their efficacy. Recent advances in intravesical drug delivery are to use hydrogels as drug reservoir to extend the drug residence time in bladder. However, because of the high viscosity of hydrogel, urinary obstruction is usually existed during the intravesical instillation. To overcome these, we developed a floating hydrogel for the delivery of Adriamycin (ADR). The floating hydrogel was made of ADR, thermosensitive polymer (Poloxamer 407) and $NaHCO_3$, which was liquid at low temperature, whereas formed gel at high temperature. In the presence of H^+, $NaHCO_3$ decomposed and produced CO_2 that attached on the surface of hydrogel and helped the hydrogel float on the urine. Hence, the urinary tract will not be blocked. Meanwhile, the encapsulated ADR released in a controlled manner. These results suggest that the floating gel may have promising applications in intravesical therapy for bladder cancer.
 J Pharm Sci 103:927–936, 2014
Keywords: intravesical instillation; drug delivery systems; controlled release; biodegradable polymers; thermal gels; cancer chemotherapy

INTRODUCTION

Bladder cancer is one of the most common malignant tumors in the urinary system, with 70% of patients presenting superficial bladder transitional cell carcinoma (TCC), which tend to recur but are generally not life threatening, and with 30% presenting as muscle-invasive bladder cancer associated with a high risk of death.[1] TCC is initially managed by transurethral resection (TUR). However, by TUR alone, TCC recurs at a rate of 50%–80% and has a 14% chance of progression to muscle-invasive cancer.[2]

To decrease TCC recurrence and progression, postoperative intravesical chemotherapy is commonly performed, which directly instill chemotherapy drugs into bladder through a urethral catheter. Intravesical instillation chemotherapy has many benefits such as high drug concentration in bladder and reduced systemic drug exposure. It can also be taken as an alternative treatment option to those who cannot receive the traditional oral administration because of systemic toxicities.[3] In the treatment, a measured amount of the drug solution is instilled to the bladder. However, the instilled drug solution cannot be held inside the bladder for more than 2 h because of the patient urination and the majority of the instilled drug is eliminated when patient voids. Especially, the residence time will be further reduced if an irritating drug is instilled into bladder as it can induce the contraction of bladder detrusor muscle. Therefore, repeated instillations are commonly required to extend the residence time of chemotherapy drug in bladder. However, repeated instillations via urethra often make patient discomfort and increase the risk of urethral infection.

Many novel intravesical instillations were reported whose main objectives were to extend the drug residence time in bladder. Thermosensitive hydrogels with sol–gel transition properties such as Poloxamer 407 (P407) attract much attention.[4,5] P407 is polyoxyethylene–polyoxypropilene–polyoxiethylene (PEOn–PPOn–PEOn) triblock copolymers, which has a molecular weight of 12,000 Da and a PEO/PPO ratio of 2:1 by weight. At concentrations of 20% or higher in aqueous solution, it exhibits the unique property of reversible thermal gelation.[6] The dominant view at present for the gelation mechanism of P407 is based on micelles packing and entanglements.[7] At low temperature, triblock copolymers form micelles, which equilibrate with Poloxamer unimers. As the temperature increases, the equilibrium shifts from unimers to spherical micelles, reducing the number of unassociated unimers in solution, leading to an increase in the micelle volume fraction. When the volume fraction is over than 0.53, the system becomes a gel by micelle packing.[8] P407 has been used as a drug reservoir to extend the drug residence time in

Abbreviations used: ADR, Adriamycin; HSA, human serum albumin; NP-ADR, Adriamycin-loaded HSA nanoparticles; NP-ADR-Gel, NP-ADR-loaded hydrogel; TCC, transitional cell carcinoma; TUR, transurethral resection; P407, Poloxamer 407; P188, Poloxamer 188; HPMC, hydroxypropyl methyl cellulose; CS, chitosan; MCC, microcrystalline cellulose; $NaHCO_3$, sodium bicarbonate.

Correspondence to: Jinhui Wu (Telephone: +86-13913026062; Fax: +86-25-83596143; E-mail: wuj@nju.edu.cn); Yiqiao Hu (Telephone: +86-13601402829; Fax: +86-25-83596143; E-mail: huyiqiao@nju.edu.cn); Hongqian Guo (Telephone: +86 13605171690; Fax: +86-25-83596143; E-mail: dr.guohongqian@gmail.com)

This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com/.

T. Lin and J. Wu authors contributed equally

Journal of Pharmaceutical Sciences, Vol. 103, 927–936 (2014)

bladder. After it forms *in situ* hydrogel, P407 can attach on the bladder wall and will not easily be eliminated by voiding, which decreases the need for repeated intravesical instillations. However, the hydrogel is easily detached from the bladder wall and possible to cause urinary obstruction.

To overcome these, a floating hydrogel delivery system was designed in this study. A mixture of P407, $NaHCO_3$, and chemotherapy drug was instilled into bladder through a urethral catheter. Because of the increased temperature in bladder, the mixture formed *in situ* hydrogel. In the acidified urine, $NaHCO_3$ decomposed and produced many CO_2 microbubbles. The produced microbubbles attached on the surface of hydrogel and helped the hydrogel float on the urine. The floating hydrogel would not block the urinary tract and might decrease the potential risk of patient urinary obstruction.

To demonstrate the potential use of the floating hydrogel in intravescial instillation, Adriamycin (ADR) was selected as the chemotherapy drug. ADR is an anthracycline antibiotic produced by the *Streptomyces* species and its main mechanisms of action is to interact with topoisomerase II.[9] Clinical trials showed that, comparing TUR alone with intravescial instillation of ADR, the average recurrence rate was 58% with TUR versus 38% with ADR, a 20% lowering of tumor recurrence.[10] However, intravesical instillation of free ADR is commonly accompanied some side effects such as bladder irritation (burning, need to urinate frequently, and pain on urination) and scarring of the bladder.[11] Therefore, in our study, ADR was first encapsulated into human serum albumin (HSA) by a folding/unfolding method we published previously.[12] Then, the optimal combination of hydrogel was screened. After that, the floating property of drug-loaded hydrogel and the cumulative release of ADR were evaluated. Finally, the *in vivo* residence time of ADR was evaluated in rats.

MATERIALS AND METHODS

Materials

Poloxamer 407, Poloxamer 188 (P188), hydroxypropyl methyl cellulose (HPMC), chitosan(CS), microcrystalline cellulose (MCC), sodium bicarbonate ($NaHCO_3$), sodium citrate, and citric acid monohydrate were purchased from Sigma–Aldrich (St. Louis, Missouri). ADR (98%, chemical grade) was purchased from Meilun Biology Technology Company, Ltd. (Dalian, Liaoning, PR China). HSA (≥96%, pharmaceutical grade) was purchased from CSL Behring GmbH (Marburg, Germany). Healthy adult female Wistar rats (6–8 weeks, 180–200 g) were obtained from the Experimental Animal Center, University of Yangzhou, China. All animal protocol was approved by Institutional Animal Care and Use Committee (IACUC) of Nanjing University.

Optimization of Hydrogel

To select the appropriate concentration, different amount of P407 were weighed and dissolved in distilled water to get the solution with the concentration of 20%, 30%, 35%, 40%, and 50% (w/v). Then, the gelation temperature and erosion time were recorded. The gelation temperature is the lowest temperature at which P407 solution coverts to gel and the erosion time is the shortest time that gel needs to dissolve in water. Then, 2% P188, 2% HPMC, 2% CS, and 2% MCC were added into P407 solution to evaluate the effects of additives on hydrogel properties. Finally, 3%, 4%, 5%, 6%, and 7% HPMC were added into P407 solution to evaluate the effects of HPMC on hydrogel properties.

Preparation of Floating Hydrogel

Floating hydrogel solution was made of $NaHCO_3$, 35% P407, and 5% HPMC. The gelation temperature and erosion time were recorded as the method mentioned above. The erosion time and the time to float were also evaluated in citrate buffer with different pH at 4.6, 5.0, 5.4, and 5.8. Time to float is the time needed from hydrogel formation to floating in water. All time were recorded with a time-counter (Thermo Fisher Scientific, Shanghai, China).

Preparation of ADR-Loaded Nanoparticles

Adriamycin-loaded HSA nanoparticles (NP-ADR) were prepared via a modified molecular switch method as described previously.[12] Briefly, 100 mg of HSA was dispersed in 50 mL of distilled water with constant stirring at 37°C, and 350 μL of β-ME was added. Three minutes later, 0.1 M NaOH solution was added to adjust the protein solution to pH 10. After that, aqueous solution of ADR·HCl (3 mL, 5 mg/mL) was added slowly to the stirred protein solution. Then, the suspension was ultrafiltrated (ultrafiltration membrane MW 30 K Da, Millipore) for five times to completely remove free ADR and β-ME and adjust the solution to about pH 7.0. After that, the NP-ADR was concentrated to 5 mg/mL (ADR) by ultrafiltration and then was freeze-dried for further use.

Incorporation of Nanoparticles in Hydrogel

A calculated amount of P407 (35%), HPMC (5%), and $NaHCO_3$(8%) was in sequence well dissolved in NP-ADR nanoparticles suspension at 4°C to form a NP-ADR-loaded hydrogel (NP-ADR-Gel) solution. The concentration of NP-ADR nanoparticles in the NP-ADR-Gel system was kept at 0.03% (w/v).

Measurements of Apparent Viscosity

The apparent viscosity was determined by using a NDJ-1 viscometer (Shanghai Balance Instrument Factory, Shanghai, China). Twenty milliliter of hydrogel solution was put in a 25-mL beaker and placed in water bath. Then, the solution was heated at a speed of 1°C/min and the viscosity was recorded every 0.5°C.

Release Study *In Vitro*

Two-hundred milliliter citric acid buffer with pH 5.0 was added in a beaker and wormed at 37°C. Twelve milliliter NP-ADR-Gel solution was kept at 4°C, and NP-ADR was injected to the citric acid buffer. At predetermined time points, 4 mL buffer was collected and equivalent volume of fresh buffer was supplied. The amount of ADR was determined by UV spectrophotometry at 480 nm.

To mimic the human urination, gel was injected in 200 mL citric acid buffer (pH 5.0). Then, the buffer was added to conical flask at the rate of 2 mL/min and the buffer was poured out when the volume was up to 400 mL. Then, the citric buffer was added at the same rate. This process was repeated until the gel was completely disappeared. At predetermined time points, 4 mL buffer was collected and equivalent volume of fresh buffer was supplied. The amount of ADR was determined by UV spectrophotometry at 480 nm.

 DOI 10.1002/jps.23854