Thermosensitive Micelles–Hydrogel Hybrid System Based on Poloxamer 407 for Localized Delivery of Paclitaxel

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ABSTRACT: A thermosensitive micelles–hydrogel hybrid system based on Poloxamer 407 (P407) was prepared to resolve the fast erosion and low loading capability of lipophilic drug of P407 gels for local chemotherapy. Different amounts of glutaraldehyde (GA) were applied to generate cross-linked networks with carboxymethyl chitosan (CMCS) interpenetrated in P407 gels, in which paclitaxel (PTX)-loaded *N*-octyl-*O*-sulfate chitosan micelles (PTX-M) were dispersed uniformly. The *in vitro* characteristics of CMCS-modified P407 gels (PTX-M-MG) were performed by examining the viscosity, swelling ratio, mechanical property, and drug release, while the *in vivo* evaluation included tissue distribution and anticancer efficacy through intratumoral administration in hepatoma solidity cell (Heps) tumor-bearing mice. The results showed that PTX-M-MG containing 0.05% (w/v) GA possessed lower viscosity, higher swelling ratio, stronger mechanical property, and longer term drug release, in which the loading efficiency of PTX was enlarged by the introduction of PTX-M. Moreover, PTX-M-MG revealed a prolonged retention at tumor sites, lasting for 20 days, and a superior tumor inhibition rate (64.27%) with reduced toxicity compared with Taxol®, PTX-M, and PTX-M loaded unmodified P407 gels (PTX-M-P407). It can be concluded that PTX-M-MG is a promising local delivery system for hydrophobic drug in cancer therapy, providing both improved efficacy and relieved side effects. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2707–2717, 2013

Keywords: cancer chemotherapy; controlled release; hydrogels; micelles; biomaterials; poloxamer 407; localized delivery

INTRODUCTION

Hydrogel has been widely used for tissue scaffolds, and drug and cell delivery systems in regenerative medicine because of its unique characteristics.[1–3] Thereinto, *in situ* gel has attracted more attention for the reason of its transition of sol to a gel state by the variation of environment, which assembles such advantages of solution and hydrogel as accurate dosage, ready to use, good compliance for patients in sol state; prolonged drug action, reduced side effects, and low frequency with which drugs need to be administered when turned into gel state.[4–7] In particular, thermosensitive hydrogel is one of the most frequently applied carriers because of the conveniently controllable adjustment of temperature.[8,9] In addition, *in situ* gel via intratumoral (i.t.) injection has been explored in cancer therapy because of the distinguished advantages of longer exposure time in tumor mass and less systemic exposure, which might enhance antitumor activity and reduce side effects.[10–12]

P407 is a hydrophilic linear triblock polymer which can be transformed from sol to a gel through micellization and gelation at a certain concentration and temperature.[13] P407 gels have been widely used in drug delivery systems, especially the local delivery, because P407 can be administered in liquid form and serve on sustained release depot at body temperature.[14,15] However, the major defect of P407 gels as a sustained release system is the rapid erosion in the physiological environment, which is induced by the macrodilution of body fluid, making the concentration of P407 to drop below the critical gelation concentration level.[16,17] In addition, the low loading capacity for poor soluble drugs of P407 gels narrows the application of drug carriers as well.[18,19] Therefore, it would be desirable to develop a P407-modified

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hydrogel possessing moderative disaggregation and high load of poor soluble drugs.

Carboxymethyl chitosan (CMCS) is a water soluble derivative of chitosan (CS) with excellent biocompatibility, which has been under investigation in a wide range of biomedical applications, such as tissue-engineering scaffolds and drug-delivery carriers.[20–22] Furthurmore, CMCS has abundant 2-NH_2 groups that can be cross-linked by glutaraldehyde (GA) to generate a network, which promotes the mechanical intensities and prevents it from the fast erosion of P407 gels.

Paclitaxel (PTX) is an effective chemotherapeutic agent by stabilized microtubules and mitotic arrest. It has shown broad-spectrum activity in several solid tumors.[23,24] Despite the clinical advances represented by PTX, it has a low therapeutic index because of the extremely hydrophobic property, which can be associated with serious side effects. PTX-loaded *N*-octyl-*O*-sulfate chitosan (NOSC) micelles (PTX-M), firstly prepared by our group, could significantly increase the water-solubility of PTX to 1000-fold with the drug-loading content about 40%, meanwhile reducing the toxicity of PTX, which is suitable for the P407 gels to improve the loading capability of PTX.[25]

Herein, we prepared an injectable micelles–hydrogel hybrid system for intratumoral drug delivery in this investigation. PTX-M was dispersed in P407 gels, in which a CMCS network was cross-linked with various concentrations of GA that interpenetrated P407 gels. The CMCS-modified P407 gels (PTX-M-MG) were developed with a view to overcome the fast dissolution and improve the mechanical strength of P407 gels, and enlarge the loading capability and extend the release period of PTX. Besides, tissue distribution and antitumor activity of PTX-M-MG after intratumoral administration were studied to further image the potential for pharmaceutical use.

MATERIALS AND METHODS

Materials

Poloxamer 407 (P407, M_w = 12, 600, PEO_{99}-PPO_{67}-PEO_{99}) was provided by Badische Anilin- and Soda-Fabrik (BASF). CMCS (carboxylation degree of 45%) was obtained from Nantong Lushen Bioengineering (Nantong, People's Republic of China). NOSC was synthesized by Zhang and group.[25,26] PTX was from Yew Pharmaceutical Company Ltd. (Jiangsu, People's Republic of China). Human hepatocyte cell line L02, and mice hepatoma solidity cell (Heps) were presented from Nanjing University, People's Republic of China. RPMI-1640 medium (Hyclone®), fetal bovine serum, penicillin–streptomycin solution (Hyclone®), phosphate buffered saline (PBS, Hyclone®), 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazoliumbromide (MTT) was provided by Sunshine Biotechnology Company Ltd. (Nanjing, People's Republic of China). Trypsin (Gibco®) was purchased from Pufei Bio-Technology (Shanghai, People's Republic of China). All other chemicals and reagents were of analytical grade. Male ICR mice (18–20 g), purchased from Nantong University (People's Republic of China), were maintained under controlled temperature and humidity conditions with free access to food and water. The experiments were carried out in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Preparation of Unloaded CMCS/GA-Modified P407 Hydrogels (P407-CMCS/GA)

The "cold method" was adopted for preparing P407 gels as described.[27] To prepare the P407-CMCS/GA with different concentrations of GA (0.025%, 0.05%, and 0.1% final concentrations, w/v), at first P407 was dispersed in the CMCS solution (P407 19%, CMCS 1.5%, w/v), and then the mixed solution (P407-CMCS) was kept at 4°C overnight to ensure the complete dissolution of P407. After that, 0.1 mL of GA solutions with different concentrations was added to 4.9 mL of P407-CMCS solutions, and eddied thoroughly to initiate the cross-linking reaction.

Measurement of Lower Critical Solution Temperature (LCST) and Gelation Time

The tube-inverting method was used to determine the sol–gel transition temperature and time.[28] The samples (1 mL) were added into 5 mL tubes (10 mm inner diameter) at 4°C and heated in a temperature-controllable water bath from 18 to 40°C at a heating rate of 1°C/min, the temperature when the liquid did not flow for 30 s was recorded as LCST. The gelation time was determined by incubating samples in a water bath at a constant temperature and inverting the tubes every 0.5 min, the time at which the liquid did not flow for 30 s was recorded as gelation time.

Rheological Studies

Rheological experiments were carried out by a rheometer (Physica MCR 301, Anton Paar, Ostfildern, Germany) with plate geometry in oscillation mode.[29] All the measurements were performed at a fixed frequency of 1 Hz and a strain of 0.1% with a gap size of 0.5 mm. To prevent the evaporation of samples, a solvent trap was used in conjunction with liquid paraffin. The viscosities of P407 and P407-CMCS solutions along with storage modulus (G′) and the loss modulus (G″) were acquired with the change of temperature at a rate of 1°C/min from the beginning temperature, 18°C. The gelation temperature was gotten when G′