Journal of Pharmaceutical Sciences 105 (2016) 194–204

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Thermosensitive Hydrogel System With Paclitaxel Liposomes Used in Localized Drug Delivery System for *In Situ* Treatment of Tumor: Better Antitumor Efficacy and Lower Toxicity

Yuling Mao [1], Xue Li [1], Ge Chen [2], Shujun Wang [3, *]

[1] School of Chinese Medicines, Shenyang Pharmaceutical University, Shenyang 110016, China
[2] Department of General Surgery, Peking Union Medical College Hospital, Beijing, China
[3] Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 11 June 2015
Revised 31 August 2015
Accepted 22 September 2015
Available online 18 November 2015

Keywords:
thermosensitive
paclitaxel liposome
intratumoral retention
pharmacodynamics
tissue distribution
biodegradable polymers
cancer chemotherapy
controlled release
injectables
lymphatic transport
thermal gels

ABSTRACT

Intratumoral delivery of chemotherapeutic agents may provide drug localization within the tumor and divert the drug from nontarget organs to improve toxicity and increase efficacy. Thermosensitive injectable hydrogel system may be suitable for the treatment of pancreatic cancer. A study was carried out to examine the efficacy and toxicity of paclitaxel (PTX) liposome gel as a local chemotherapy system against pancreatic cancer in tumor-bearing mice model. The thermosensitive hydrogel we prepared had an appropriate sol-to-gel transition temperature and particle size and morphology study showed this new dosage form possessed physical stability of drug without precipitation and particle size growth of liposome. PTX-lip-gel release *in vitro* showed a much more slowly release than PTX-lip. The PTX-lip-gel system was proven to have a good retention inside of tumor tissue by intratumoral retention experiments. The *in vivo* trials showed a better balance between antitumor efficacy and systemic safety in PTX-lip-gel group than in other groups at the equal drug dose. In conclusion, the PTX-lip-gel we prepared in this study provided a high local PTX concentration, sustained and stable drug release, extend drug retention inside of tumor, and low toxicity to normal tissues.

Introduction

Pancreatic cancer is a lethal human malignancy with an all-stage 5-year survival frequency of less than 5%, which highlights the urgent need for more effective therapeutic strategies.[1] To overcome issues with pancreas-targeted therapeutic, we designed an *in situ* injectable, thermosensitive hydrogel which paclitaxel liposomes (PTX-lip) were incoperated into (Fig. 1). For this hydrogel, its ability to delivery chemotherapeutic agents intratumorally or intralesionally has been explored as a potential strategy to maximize antitumor effect, reduce systemic toxicity providing a continuous and sustained drug delivery.[2] Intratumoral delivery of chemotherapeutic agents may provide drug localization within the tumor and divert the drug from nontarget organs to improve toxicity and increase efficacy.[3] Local chemotherapy represents a growing trend for maximizing local tumor control with minimal systemic toxicity.[4,5] Thermosensitive injectable hydrogel systems have gained attention because of their noninvasiveness, compared with the other localized implantable systems, with the ability to carry therapeutic agents for site-specific delivery, prolonged drug action, improved patient compliance, and reduced systemic toxicity.[6,7] Parenteral applications of *in situ* gelling thermosensitive poloxamer systems, especially subcutaneous and intratumoral injection, seem to be the ones most studied. An appropriate delivery matrix for drugs or drug-loaded particles is sought for over the last 20 years in order to develop prolonged drug-release formulations.

Poloxamer 407 (P407), known under the registered trademark of Pluronic F127 (BASF Laboratories, Wyandotte, Michigan), and poloxamer 188 (P188) are most widely studied as temperature-sensitive polymers and have been approved by the US FDA. They are commercially available and considered to be nontoxic. The P407 hydrogel-added P188 is based on the ordered packing.[8] P407 had been intensively investigated for various routes of application such as ocular, nasal, vaginal, rectal, buccal, topical, transdermal, and parenteral injections. It is a promising approach for the treatment

This article contains supplementary material available from the authors upon request or via the Internet at http://dx.doi.org/10.1002/jps.24693.

* *Correspondence to*: Shujun Wang (Telephone: +86-024-23986360; Fax: +86-024-23986360).
E-mail address: wangshujun2015@hotmail.com (S. Wang).

http://dx.doi.org/10.1002/jps.24693
0022-3549/

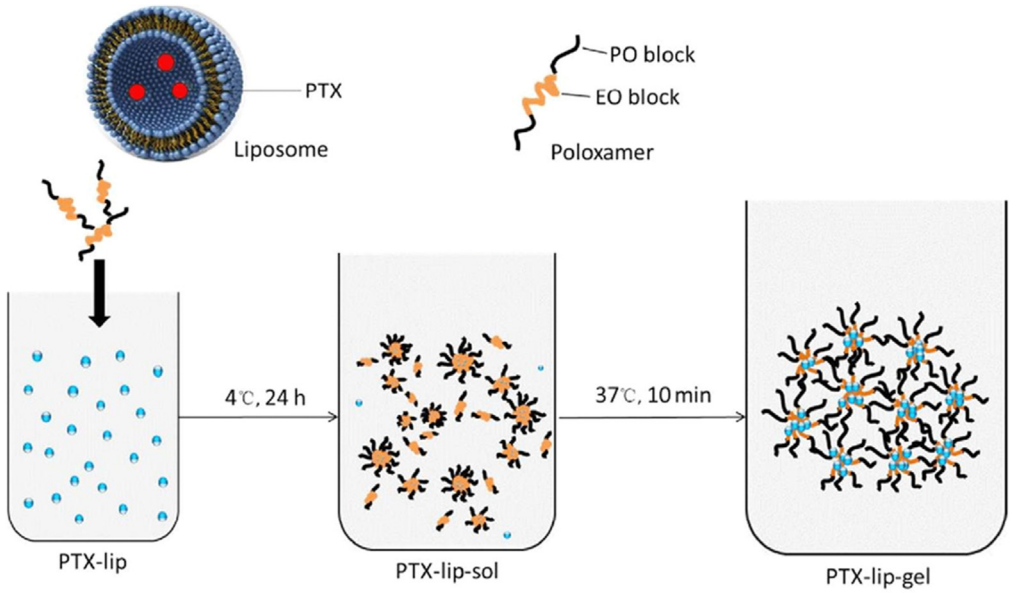

Figure 1. The loading process of PTX liposome in hydrogel.

of solid tumors accessible in neo-adjuvant or adjuvant settings.[8,9] A liposome is an artificially prepared spherical vesicle composed of a lamellar phase lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents.

Anticancer drugs with severe acute toxicity such as PTX may be good candidates for using this hydrogel formulation for improving efficacy. PTX is a cytotoxic drug used for the treatment of several solid tumors. However, tumor localization and cardiac toxicity of PTX-lip can still be improved. Therefore, we hypothesized that a combination drug delivery system based on *in situ* gel entrapment of drug liposome can prolong drug release, enhance PTX-lip's antitumor effect by *in situ* treatment and achieve lower toxicity, especially cardiac toxicity.

Materials and Methods

Materials

The following materials were purchased from the sources in parentheses: PTX (Haikou Pharmaceutical Company, Ltd., Hainan, China); internal standard docetaxel (Chinese National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China); P407 and 188 (BASF AG, Ludwigshafen, Germany); methanol, acetonitrile, and dehydrated alcohol (Tianjin Concord Technology Company Ltd., Tianjin, China); soybean phospholipid (Shanghai Advanced Vehicle Technology Company Ltd., Shanghai, China), cholesterol (Tianjin Bodi Chemical Company Ltd., Tianjin, China); and Cy7 (710/790 nm) (Beijing Fanbo Biochemicals Company Ltd., Beijing, China). All chemicals and reagents used were of analytical or chromatographic grade.

The mouse S180 ascitic tumor cell line was obtained from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. These cells were maintained and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. All the cell culture media contained 100 μg/mL gentamicin and were cultured at 37°C with 5% CO_2.[10]

KM mice, male, 18-22 g male KM mice (20 ± 2 g) were provided from the Laboratory Animal Center, Shenyang Pharmaceutical University (Shenyang, China). Upon arrival, the mice were housed in room temperature and humidity, and had access to water and food *ad libitum*. All the experimental protocols adhered to the principles of laboratory animal care and were approved by the Experimental Animal Use and Care Committee, Shenyang Pharmaceutical University.

Preparation of Taxol ®, PTX-Lip, PTX-Lip-Gel, Blank Gel, Cy7-Lip, and Cy7-Lip-Gel

Paclitaxel Liposome

Paclitaxel liposomes were prepared by the carrier-deposition method according to our previous study.[11] Briefly, a mixture of SPC/cholesterol/PTX (35/26/4, by mM) were dissolved in anhydrous ethanol, then the resultant solution and 1.50 g of 100 mm dry dextrose powder were mixed and dried to freely flow by a rotary evaporator (RE52CS; Shanghai Yarong Bio-Chem Instruments, China). The dry powder of PTX-lip was reconstituted to form the liposomes solution by the following method: 10 mL pH7.4 phosphate-buffered saline (PBS) was placed into the dry powder with magnetic stirring at 50 rpm. The reconstituted PTX-lip solution was sonicated (400 W, 5 min), followed by using an Avanti Mini Extruder (Avanti Polar Lipids) to extrude through polycarbonate membranes with 0.08 mm pore sizes.[12]

Blank Gel

Blank gel was prepared by adding 2 g P407 and 0.5 g P188 into 7.5 mL deionized water with final concentrations of P407 20% and P188 5%. The mixture was then stirred with the magnetic stirrers until all of the P407 and P188 granules were completely dissolved and stored in 4°C for 24 h until a clear solution was obtained.

Paclitaxel Liposome Gel

Paclitaxel liposome gel was prepared according to the “cold” method as previously described.[13,14] Appropriate amount of P407