European Journal of Pharmaceutics and Biopharmaceutics 93 (2015) 127–135

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research Paper

The use of cisplatin-loaded mucoadhesive nanofibers for local chemotherapy of cervical cancers in mice

Shan Zong [b,1], Xue Wang [c,1], Yongping Yang [b], Wenbin Wu [d], Hongjun Li [c], Yue Ma [b], Wenhai Lin [a], Tingting Sun [a], Yubin Huang [a], Zhigang Xie [a], Ying Yue [b,*], Shi Liu [a,*], Xiabin Jing [a]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[b] The First Hospital of Jilin University, Changchun 130021, China
[c] China-Japan Union Hospital of Jilin University, Changchun 130033, China
[d] Xuzhou Central Hospital Affiliated to Southeast University, Xuzhou 221009, China

ARTICLE INFO

Article history:
Received 5 November 2014
Revised 22 March 2015
Accepted in revised form 30 March 2015
Available online 3 April 2015

Keywords:
Cervical cancer
Nanofibers
Cisplatin
Vaginal delivery
Local chemotherapy

ABSTRACT

Polymer-based local drug delivery system may be suitable for the treatment of cervix cancer. A pilot study was carried out to examine the efficacy of cisplatin-loaded poly(ethylene oxide)/polylactide composite electrospun nanofibers as a local chemotherapy system against cervical cancer in mice *via* vaginal implantation. The nanofibers were proven to have good mucoadhesive property by *in vitro* mucoadhesion test and *in vivo* vaginal retention evaluation. An orthotopic cervical/vaginal cancer model was established by injecting murine cervical cancer U14 cells into the vaginal submucosa nearby the cervix. By inserting the nanofibers mat into the vagina of mice, the cisplatin released from the fiber-mat showed a much more accumulation in the vagina/cervix region than in the peripheral organs such as kidneys, liver, or blood, in contrary to the case of intravenous (i.v) injection. The *in vivo* trials showed that a better balance between anti-tumor efficacy and systemic safety was achieved in nanofibers group than that in i.v injection group at the equal drug dose. Therefore, electrospun nanofibers present a promising approach to the local drug delivery *via* vagina against cervical cancer.

1. Introduction

Cervical cancer is a leading cause of death by cancer among women worldwide, with approximately half a million new cases and over 200,000 deaths each year. Patients with high grade cervical cancer have extremely poor survival rates, resulting in the high numbers of deaths among those afflicted with this cancer [1].

For local advanced cervical cancers, a combination of radiation therapy and chemotherapy is usually applied to increase survival rates of patients. Concurrent cisplatin chemotherapy has long been used in clinic practice as an adjuvant to radiation therapy. However, the lack of selectivity for tumor tissues often leads to some uncomfortable adverse effect for the patients who undergo a chemotherapeutic procedure, including kidney malfunction, nausea and vomiting, nerve damage, impairment of sight, and suppression of the bone marrow. [2,3].

The location of the cervix allows for easy accessibility and the vagina is a favorable site for local and systemic delivery of drugs. To date, a number of vaginal drug delivery systems for contraception and vaginal infections have been utilized in clinical and research settings, including gels [4], tablets [5], intravaginal rings (IVRs) [6] and films [7]. Theoretically, they are also suitable for the treatment of cervical cancer but with some disadvantages. For example, vaginal gels are mainly suitable for hydrophilic drugs and can easily leak out of the vagina. Other formulations also face variant problems such as poor vaginal residence time for the tablets [8], low drug-loading rate for the film [9] or high prices for IVRs [10], which more or less limit their application in the treatment of cervical cancer.

Recently, drug-eluting electrospun nanofibers as a novel dosage form have attracted much research interest because they exhibited following advantages: high loading and encapsulation efficiency for physicochemically diverse drugs; relatively prolonged residence time; desirable distribution and delivery of the active substance for an extended period at a predictable rate; typical softness, flexibility, non-abrasion and lack of sharp corners which enable them realize various geometries (sheets, tubes, coatings) to fit the lesions; much less cost and easy operation [9–14].

* Corresponding authors. Tel./fax: +86 0431 81875321 (Y. Yue). Tel./fax: +86 0431 85262940 (S. Liu).
E-mail addresses: yying119@126.com (Y. Yue), liushi@ciac.ac.cn (S. Liu).
[1] The first two authors of equal contribution.

http://dx.doi.org/10.1016/j.ejpb.2015.03.029
0939-6411/

Therefore, there is a good reason to use drug-loaded nanofibers to treat cervical cancers locally *via* the vagina.

In the present study, therefore, cisplatin-loaded poly(ethylene oxide)/polylactide composite electrospun nanofibers were prepared, followed by the evaluation of the mucoadhesion, *in vitro* and *in vivo* release profile and bio-distribution. The orthotopic cervical cancer model in mice was established and used for the assessment of the efficacy and safety of the drug-loaded nanofibers. The purpose of our study was to seek the possibility and feasibility of nanofibers-based vaginal drug delivery system for local chemotherapy against the cervical cancer.

2. Materials and methods

2.1. Materials and animals

Poly(L-lactide) (PLA) was synthesized from L-lactide in our laboratory. Its molecular weight (Mn) and polydispersity (PD) determined by GPC were 138 kg mol^{-1} and 3.41, respectively. Poly(ethylene oxide) (PEO, Mw = 100 kg mol^{-1}) was purchased from Alfa Aesar Co. Ltd. (Tianjin, China). Fluorescein sodium salt (coded as "Flu") and hydroxypropyl methyl cellulose (HPMC, viscosity = 100 Pa s) were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Cisplatin (>99.0%, coded as "CDDP") was purchased from Boyuan Pharmaceutical Co. Ltd. (Shandong, China).

Female Kunming mice with body weight ranging from 25 to 40 g were provided by the Experimental Animal Center of Jilin University.

2.2. Preparation and characterization of cisplatin-loaded nanofibers

A 1:2 (wt/wt) mixture of PLA and PEO was dissolved in 2,2,2-trifluoroethanol to form a 10% (wt/vol.) solution, followed by the addition of cisplatin/dimethylsulfoxide (DMSO) solution with a weight ratio of cisplatin to polymer of 10%. Electrospinning parameters were as follows: electric field strength: 1.8–2.0 kV cm^{-1}; air gap distance: 15 cm; inner diameter of spinneret: 0.4 mm; flow rate of solution: 1–2 ml h^{-1} and all the experiments were conducted at room temperature in air. The cisplatin-loaded composite nanofibers were coded as $CDDP_{10}/PLA_1$-PEO_2 fiber or simply CDDP/fiber. As a control, unloaded PLA_1-PEO_2 fibers were prepared.

The preparation protocol of 2% Flu-loaded composite nanofibers was similar to that of CDDP/fiber and the fiber-mat collected was coded as Flu_2/fiber. Meanwhile, 2% Flu-loaded HPMC gel and 2% Flu-loaded HPMC film were prepared as described in the previous Refs. [15,16]. Briefly, 2% (w/v) of HPMC K100M was dispersed in deionized water containing Flu (2 wt% of HPMC used) by stirring at 500 rpm for 60 min at room temperature and the gel collected was coded as Flu_2/HPMC gel. For HPMC film, a 1% (w/v) Carbopol–HPMC solution containing Flu (2 wt% of polymer used) was allowed to stir for 10 h and was poured into a Petri dish and dried in the oven at 60 °C for 24 h. The films were carefully peeled off and coded as Flu_2/HPMC film.

An environmental scanning electron microscope (ESEM, Model XL 30 ESEM FEG from Micro FEI Philips) was used to observe the morphology of CDDP/fiber. Water contact angles of the CDDP/fiber were monitored with a video contact-angle instrument (DSA100 Kruss GmbH, German) to assess the wettability of the fiber-mat, As 2 μl of deionized water was automatically dropped onto the fiber-mat, the contact angle was determined within 40 s.

2.3. The studies of mucoadhesive properties and release behavior in vitro

A simulated dynamic vaginal system (Fig. 1) was prepared by assembling a slide tube and a peristaltic pump using the method previously reported [16]. Before the experiments, rat vaginal tissue which has been cut into pieces of 2 cm^2 in size was mounted with the mucosa side up on a cylindric glass slide (60° slope). The CDDP/fiber of 1 cm^2 in size was mounted on the mucosal membrane. Sodium acetate buffer (pH 5.0) was allowed to flow through the vaginal tissue with a flow rate of 10 ml h^{-1} and finally collected into a receptor beaker placed under the slide tube for 24 h. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the amount of released cisplatin in the collected buffer solution, residual cisplatin remaining in the fiber-mat, and absorbed cisplatin in the mucosal tissue.

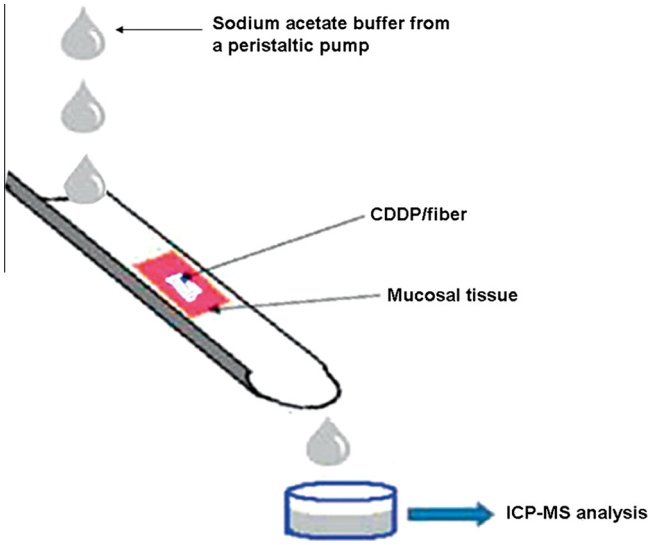

Fig. 1. Flow-through experimental setup for evaluating mucoadhesive properties and *in vitro* release profile.

Meanwhile, residence time of the fiber-mat on the mucosa was also measured using the same system.

2.4. Measurement of retention of fiber-mat in vagina

Two sets of experiments were performed. In the first one, three female KM mice were used to measure the vaginal retention of Flu_2/fiber, Flu_2/HPMC film and Flu_2/HPMC gel.

Both fiber-mat and film of 50 mm^2 in size were rolled into a tube and inserted into the mouse vagina. About 0.1 ml of gel was injected into mouse vagina by a syringe. After administration, each mouse was immediately kept in an overturned beaker for 5 min (gel) or 1 h (film and fiber-mat) with a black plate below the beaker to collect possible leakage. Then the black plate was imaged by fluorescent imaging system (CRI Maestro 500FL, USA) to detect leaked Flu signals.

In the second one, 10 female mice were implanted with CDDP/fiber of 50 mm^2 in size. Then each mouse was put into an individual bedding-free cage to observe the leakage occurrence of the fiber-mat. Three days after implantation, all mice were checked whether there was residual fiber-mat in the vagina.

The study protocol was approved by the local institution review board and performed according to the Guidelines of the Committee on Animal Use and Care of Chinese Academy of Sciences.

2.5. Cervical cancer models

Mouse uterine cervical cancer U14 cell line was purchased from the Medical Department of Jilin University in China. Female KM mice were used to prepare the cervical cancer models. U14 cells (2×10^7 cells/ml) in PBS (25 μl) were injected into vaginal