Available online at www.sciencedirect.com
ScienceDirect
Journal of Controlled Release 114 (2006) 307–316
journal of controlled release
www.elsevier.com/locate/jconrel

BCNU-loaded PEG–PLLA ultrafine fibers and their in vitro antitumor activity against Glioma C6 cells

Xiuling Xu, Xuesi Chen, Xiaoyi Xu, Tiancheng Lu, Xin Wang, Lixin Yang, Xiabin Jing *

State key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
Graduate School of Chinese Academy of Sciences, Beijing, 100039, China

Received 6 January 2006; accepted 29 May 2006
Available online 23 June 2006

Abstract

The purpose of the present study was to develop implantable BCNU-loaded poly(ethylene glycol)–poly(L-lactic acid) (PEG–PLLA) diblock copolymer fibers for the controlled release of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). BCNU was well incorporated and dispersed uniformly in biodegradable PEG–PLLA fibers by using electrospinning method. Environmental Scanning Electron Microscope (ESEM) images indicated that the BCNU-loaded PEG–PLLA fibers looked uniform and their surfaces were reasonably smooth. Their average diameters were below 1500 nm. The release rate of BCNU from the fiber mats increased with the increase of BCNU loading amount. In vitro cytotoxicity assay showed that the PEG–PLLA fibers themselves did not affect the growth of rat Glioma C6 cells. Antitumor activity of the BCNU-loaded fibers against the cells was kept over the whole experiment process, while that of pristine BCNU disappeared within 48 h. These results strongly suggest that the BCNU/PEG–PLLA fibers have an effect of controlled release of BCNU and are suitable for postoperative chemotherapy of cancers.

Keywords: BCNU; PEG–PLLA fibers; Electrospinning; Cytotoxicity

1. Introduction

BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) is one of the most widely used antineoplastic agents for the treatment of malignant gliomas [1]. It can penetrate blood–brain barrier at potentially tumoricidal concentrations, because of its good lipid solubility and relatively low molecular weight [1]. It is generally accepted that its action mechanism is the formation of interstrand cross-links in DNA, RNA and protein similar to other alkylating agents [2–4].

The conventional method of delivering BCNU to pathological site is mainly through intravenous perfusion [5]. However, this form of therapy leads to severe side effects, including bone marrow suppression, hepatic dysfunction and pulmonary fibrosis due to the toxicity of the drug [6]. Furthermore, when given intravenously, BCNU has a plasma half-life of only about 20 min *in vitro* and less than 15 min *in vivo*, which further limits its efficacy after systemic application [1]. One promising approach to overcome these disadvantages is the localized and controlled delivery of BCNU using biodegradable polymeric release matrices implanted in the tumor bed. The advantages of this method are not only achievement of a high local drug concentration by using a small amount of drug, but also minimization of severe side effects. Moreover, the local sustained release of BCNU potentially increases the duration of tumor exposure to the drug.

The polyanhydride poly[bis(carboxyphenoxy-propane)–sebacic acid] (PCPP–SA) matrix is an example of a biodegradable polymer that has been clinically investigated for glioma therapy [7]. However, the maximum release period using a PCPP–SA copolymer (CPP/SA = 50:50) was 18 days after an initial burst release of BCNU within first 24 h [8–11]. Another attempt is the preparation of BCNU-loaded poly(L-lactide-co-glycolide) (PLGA) microparticles by spray-drying method [12,13]. But quite an amount of the drug is lost during the spray-drying process and the microparticles are mostly aggregated and even lose their spherical shape with the increase of BCNU loading amount. Recently, Jin Soo Lee et al. [14] have fabricated BCNU-containing wafers by compression molding of BCNU and PLGA mixtures without using organic solvent. Unfortunately, relatively

* Corresponding author. Tel./fax: +86 431 5262112.
E-mail address: xbjing@ciac.jl.cn (X. Jing).

0168-3659/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2006.05.031

high burst release at the beginning was observed during the *in vitro* release study. For example, about 40% BCNU is released from 10% BCNU-loaded PLGA wafer in the first 6 h while 100% BCNU release needs almost 7 days.

In recent years, with the development of electrospinning, the use of electrospun fibers as drug carriers seems to be a promising method for delivering the anticancer drugs, especially in post-operative local chemotherapy, because they have numerous advantages, such as improved therapeutic effect, reduced toxicity, and handling convenience. In a typical eletrospinning process, a strong electrostatic field is applied to a polymer solution held in a syringe with a capillary outlet. When the surface tension of the polymer solution is overcome by the electric force, a fiber is extruded from the outlet. This unique and useful technique can produce ultrafine fibers with diameters ranging from several microns down to less than 100 nm [15]. In addition, the ultrafine fiber mats have large specific surface area and look like absorbent cotton or paper and can be used easily.

In this study, to investigate the utility of biodegradable polymer fiber carrier for long-term delivery of BCNU, the BCNU-loaded polymer fibers were prepared via electrospinning. The distribution of BCNU in the fibers was examined and *in vitro* release profile and antitumor activity of the BCNU-containing fibers were investigated.

2. Experimental section

2.1. Materials

BCNU was purchased from Dalian Hongfeng Pharmaceutical Co. Ltd. and stored at −20 °C. RPMI 1640 (the culture medium) and calf serum were supplied by Gibco (Grand Island, NY). Triethyl benzyl ammonium chloride (TEBAC) and MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide) were obtained from Sigma and were used without further purification.

The random copolymer PLGA (poly(L-lactide-co-glycolide), molar ratio of lactide to glycolide 80:20) and diblock copolymer PEG–PLLA (prepared from PEG750 and lactide) were synthesized in our lab. Their molecular weight and polydispersity (Mn/PD) determined by GPC were 60,800/2.88 and 84,800/3.41, respectively.

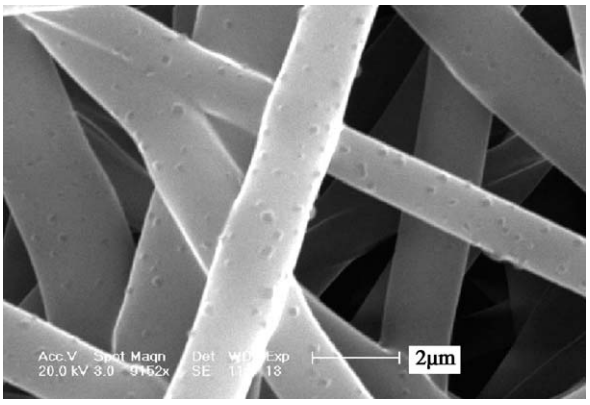

Fig. 1. ESEM photograph of 25 wt.% BCNU/PLGA (80:20) fibers.

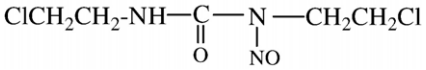

Fig. 2. Molecular structure of BCNU.

2.2. Preparation of BCNU-loaded fiber mats

PLGA and PEG–PLLA were dissolved in chloroform to prepare a 12 wt.% and 7.5 wt.% solution, respectively. 5 wt.% of TEBAC and 5–20 wt.% of BCNU with respect to the polymer used were added into the polymer solution. The solution was then immediately electrospun.

The electrospinning set-up was described by Zeng et al. [16]. The polymer and BCNU blend solution was transferred to a 30 ml syringe with a right angle-shaped needle of 0.4 mm in inner diameter attached to it. A pressure was applied to the solution in syringe to maintain a steady flow of the solution from the needle outlet in the range of 2–2.5 ml/h. The electric field strength was 1.46 kV/cm. The distance between the needle tip and the grounded target was 24 cm. All electrospinning experiments were carried out at about 20 °C in air. In order to remove the residual chloroform, the fiber mats collected were freeze-dried for about 48 h at −50 °C under a vacuum of 10 Pa. The composite fibers obtained are abbreviated as BCNU/PEG–PLLA hereafter for simplicity.

2.3. Characterization of BCNU-loaded fiber mats

An environmental scanning electron microscope (ESEM, Model XL 30 ESEM FEG from Micro FEI Philips) equipped with an EDS accessory was used to observe both the morphology and nitrogen elemental distribution in the fiber.

The crystalline states of BCNU, PEG–PLLA, and BCNU/PEG–PLLA fiber mats were analyzed by wide angle X-ray diffraction (WAXD, Rigaku, D/max 2500V PC). The samples were scanned from 5° to 60° at a scanning rate of 5°/min.

Thermal parameters such as melting temperature (T_m) of BCNU, glass transition temperatures (T_g) of the PEG–PLLA and BCNU/PEG–PLLA fiber mats and crystallinity of PEG–PLLA were determined by differential scanning calorimetry (DSC-7, from Perkin Elmer) at a heating rate of 10 °C/min from 0 to 200 °C under a N_2 atmosphere. Crystallinity of PEG–PLLA in the composite fiber mats was calculated from the following formula:

$$X_{\mathrm{PLLA}}\ (\%) = (\Delta H_m/93.7/W_{\mathrm{PLLA}}) \times 100\%$$

where ΔH_m stands for the melting enthalpy in J/g that was calculated from the fusion peaks in the first heating run of DSC curve, 93.7 (J/g) is the theoretical fusion enthalpy of completely crystalline PLLA [17], and W_{PLLA} is the weight fraction of the PLLA block in the copolymer.

2.4. In vitro drug release

A piece of BCNU-loaded fiber mat (20–30 mg, 2 cm × 1.5 cm × 0.25 mm) was placed in a vial filled with 25 ml of