Journal of Photochemistry and Photobiology B: Biology 149 (2015) 51–57

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Preparation and characterization of injectable Mitoxantrone poly (lactic acid)/fullerene implants for in vivo chemo-photodynamic therapy

Zhi Li, Fei-long Zhang, Li-li Pan, Xia-li Zhu, Zhen-zhong Zhang *

School of Pharmaceutical Sciences, Zhengzhou University, 100 Kexue Avenue, Zhengzhou 450001, PR China

ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form 21 May 2015
Accepted 21 May 2015
Available online 27 May 2015

Keywords:
Fullerene (C60)
Mitoxantrone
Poly (lactic acid)
Chemo-photodynamic therapy
Implants
Sustained release

ABSTRACT

Fullerene (C60) L-phenylalanine derivative attached with poly (lactic acid) (C60-phe-PLA) was developed to prepare injectable Mitoxantrone (MTX) multifunctional implants. C60-phe-PLA was self-assembled to form microspheres consisting of a hydrophilic antitumor drug (MTX) and a hydrophobic block (C60) by dispersion–solvent diffusion method. The self-assembled microspheres showed sustained release pattern almost 15 days in vitro release experiments. According to the tissue distribution of C57BL mice after intratumoral administration of the microspheres, the MTX mainly distributed in tumors, and rarely in heart, liver, spleen, lung, and kidney. Photodynamic antitumor efficacy of blank microsphere was realized. Microspheres afforded high antitumor efficacy without obvious toxic effects to normal organs, owing to its significantly increased MTX tumor retention time, low MTX levels in normal organs and strong photodynamic activity of PLA-phe-C60. These C60-phe-PLA microspheres may be promising for the efficacy with minimal side effects in future treatment of solid tumors.

1. Introduction

Cancer is a major cause of death in human throughout the world. Current clinical strategies for treating cancer include surgery, radiation therapy, chemotherapy, and more recently, photodynamic therapy (PDT). PDT involves three components: a photosensitizer (PS), a special wavelength of drug-activating light and oxygen. A major advantage of PDT over conventional chemotherapy is that PS itself is minimally toxic in the absence of light. Furthermore, compared with radiotherapy, the activating light is non-ionizing and hence its effect on tissues without PS drug is not harmful [1–3]. Fullerene (C60), the third allotrope of carbon, are nano-scale carbon materials with unique photo-, electro-chemical, physical properties and low systemic toxicity [4–7]. In addition, C60 possess unique photochemical properties: under the irradiation of UV or visible light, C60 molecule is able to shift to the excitation triplet state and generate singlet and other active forms of oxygen [8,9]. Due to the enormous PDT potential of fullerene, there has been much interest in studying possible biological activities of fullerenes to use them as PS in medicine, for example, malonic acid derivatives of C60 have a obvious photosensitive effect and significant cytotoxicity in vitro experiments [10]. Recently, we reported the photocytotoxicity of fullerene aminoacid derivative, revealed that p38MAPK is activated by C60 nanoparticles through triggering reactive oxygens species generation, leading to cancer cell injuries in vitro [11]. In this work, PDT was conducted to obtain a synergistic effect on malignant tumor in C57 mice models by use of the C60 based drug delivery system.

In most current clinical application, the PS is formulated in lipidic or organic excipients and given as an intravenously injection leading to unpredictable biodistribution [12,13]. While, biodegradable implants containing chemotherapeutic agents are clinically useful for both site-specific and systemic drug therapies in cancer. By intratumoral administration of an implant, it is possible to retain the drug in tumor at cytotoxicity levels for a long time and overcome the drawbacks, such as toxicity, metabolic deactivation and frequent, repeated intake of medicines [14,15]. Poly (lactic acid) (PLA), a biocompatible and biodegradable copolymer, has been mostly used for delivering drugs [16–18]. In this study, a fullerene L-phenylalanine derivative was synthesized, then fuctionalized by PLA, giving C60-phe-PLA with self-assembled ability, and then prepared microspheres consisting of Mitoxantrone (MTX) by dispersion–solvent diffusion method, in order to get a system for combination chemotherapy with PDT.

MTX is an anthracycline anticancer drug. It has been effectively used in the treatment of ovarian and hepatic cancer, breast cancer, lymphoma and leukemia [19–21]. Although MTX has low toxicity, high doses of MTX or its frequent administration may cause lots

* Corresponding author.
E-mail address: zhenzhongz@126.com (Z.-z. Zhang).

http://dx.doi.org/10.1016/j.jphotobiol.2015.05.018
1011-1344/

potential toxic effects, one of the most serious adverse events associated with MTX treatment is cardiotoxicity [22,23].

Herein in this study, injectable C60-phe-PLA /MTX implants (Fig. 1) were developed and characterized by transmission electron microscopy (TEM), dynamic laser scattering (DLS), scanning electron microscopy (SEM) and its photodynamic efficacy, MTX release efficiency and its treatment effect in vivo were examined using melanoma tumor-bearing mice models.

2. Materials and methods

2.1. Materials

Fullerene (C60, purity 95%) were purchased from Henan Fengyuan Chemicals Co. Ltd. Mitoxantrone HCl (MTX, 20120503, purity 98%) was gotten from Beijing Yi-He Biotech Co. Ltd.

Poly (lactic acid) (PLA, average molecular weight 10,000–18,000) was obtained from Sigma–Aldrich Co. LLC. Tin protochloride ($SnCl_2$) was purchased from Aladdin chemistry Co. Ltd. Sulforhodamine B (SRB), DMEM cell culture medium, penicillin, streptomycin, fetal bovine serum (FBS) were bought from Gibco Invitrogen. L-phenylalanine (phe) and other reagents were acquired from China National Medicine Corporation Ltd.

2.2. Synthesis of C60-phe-PLA and characterization

C60 Phenylalanine derivative (C60-phe) was synthesized according to the procedure of our previous study [11]. C60-phe was attached with PLA through ester bonds. C60-phe (100 mg) was suspended with dl-lactic acid (1 ml), 1% (w/w) $SnCl_2$, PLA (1 g) was then added. The mixture was allowed to react at 110 °C for 1 h under N_2 and then stirred at 140 °C for 2 h. After the conjugate was dissolved in dichloromethane, then the conjugate was precipitated using deionized water. The precipitated was purified by repeated rinsing with deionized water and filtrations to remove the unreacted reagents to obtain C60-phe-PLA complex.

TEM (Tecnai G2 20, FEI) were used for morphological of C60-phe-PLA and C60-phe-PLA was dispersed in dichloromethane. FT-IR spectra were recorded in KBr pastilles on a Nicolet iS10 spectrometer (Thermo). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance DPX300 (300 MHz) Fourier-transform NMR spectrometer (Bruker, Fallanden, Switzerland) from solutions in Dichloromethane-d2. The relative amount of PLA linked to C60-phe was tested using a thermal gravimetric analysis (TGA, PerkinElmer) with the experimental conditions of scanning from 25 to 800 °C under nitrogen at a heating rate of 20 °C/min.

2.3. Microsphere formulation and characterization

C60-phe-PLA (200 mg) was dissolved in 3 ml of dichloromethane. Then MTX saturated aqueous solution (1 ml) was added. The mixture solution was stirred at room temperature for 30 min. After that, the solution was dropped into 40 ml of glycerol by stirring at 1800 rpm for 10 min in an ice bath. The microsphere glycerol solution was dispersed in 5% (w/v) gelatin solution by stirring at 700 rpm for 2 h. The resulting microspheres were separated by filtration and then dried in vacuum at 50 °Cfor 24 h. Blank microspheres were prepared following the same procedure, but without adding MTX to C60-phe-PLA solution.

Microspheres morphology was assessed with scanning electron microscopy (SEM). Dynamic lights scattering (DLS) measurements of particle size were carried out using a Zetasizer Nano-ZS90 light scattering instrument (Malvern Instruments, Enigma Business Park, UK).

MTX content was determined by the following method. An accurately weighed amount of microspheres (about 10 mg) was dissolved in 3 ml of DMSO, the solution was centrifuged to remove precipitated polymer, and the drug concentration was analysed at 627 nm by UV spectrometer.

2.4. In vitro release studies

Two groups were designed, one for 532 nm/laser (Diode laser, CW, Changchun laser research center, 100 mW/cm^2, 1 min) everyday, the other without laser. The release experiments were conducted at 37 °C. 10 mg dried microspheres were accurately weighed and re-suspended in 20 ml release medium (PBS, pH 6.8), then was incubated at 37 °C with a shaking speed 100 rpm. At each specified intervals, 1 ml solution was drawn from the release medium, being replaced by the same volume of fresh PBS. The concentration of MTX released from microspheres into PBS solution was quantified using high performance liquid chromatography (HPLC, 1100 Agilent, USA) with the following conditions: an Eclipse XDB-C18 column (150 mm × 4.6 mm, 5.0 μm); mobile phase acetate/methanol 64:36; column temperature 30 °C; detection wave length 627 nm; flow rate 1.0 ml/min.

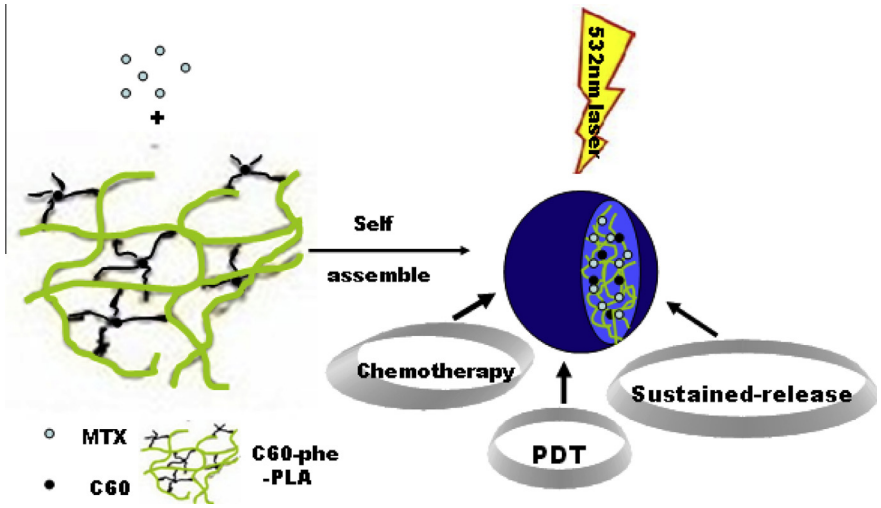

Fig. 1. Scheme of fullerene-based multi-functional sustained-release microsphere and its bio-functions.