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In vitro study of anticancer drug doxorubicin in PLGA-based microparticles

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Abstract

Doxorubicin (DOX), also known as adriamycin, is an anthracycline drug commonly used in cancer chemotherapy. Unfortunately, its therapeutic potential has been restricted by its dose limited cardiotoxicity and the resistance developed by the tumor cells to the molecule after some time of treatment. One way to overcome these problems is to encapsulate the drug in poly (D, L-lactide-*co*-glycolide) (PLGA) microparticles. This paper investigates the release characteristics of DOX from polymeric carriers fabricated using the spray-drying technique. The encapsulation efficiency, size and morphology of the various polymeric devices were also determined. In order to improve the release characteristics, Pluronic P105 (PLU) and poly (L-Lactide) (PLLA) are individually used in combination with PLGA. Finally, a cytotoxicity test was performed using Glioma C6 cancer cells to investigate the cytotoxicity of DOX delivered from PLGA microparticles. It has been found that the cytotoxicity of DOX to Glioma C6 cancer cells is enhanced when DOX is delivered from PLGA polymeric carrier.

Keywords: Microparticles; Doxorubicin (DOX); Poly(D,L-lactide-*co*-glycolide) (PLGA); Cytotoxicity; Spray-drying

1. Introduction

Doxorubicin (DOX), an anthracycline antibiotic, is one of the most important cytostatic drugs used in the field of cancer chemotherapy [1–3]. It works by interfering with the growth of rapidly growing cancer cells where it binds and intercalates into the DNA strand, thus, inhibiting further DNA and RNA biosynthesis, eventually causing cell death [2,4,5].

DOX is usually used in the treatment of neoplastic diseases, such as leukemia and various solid tumors. It is particularly effective in the treatment of breast cancer and is the protocol therapy for AIDS related Kaposi's sarcoma. It also has notable activity against tumor of the ovaries, lung, testes, prostate, cervix, bladder and Ewing's sarcoma. Unfortunately, like many other drugs used to treat cancer, DOX is a potent vesicant that may cause extravasations and necrosis at the injection site or any site that the skin is exposed to. Hence, in this paper, we propose the use of a biodegradable polymer matrix, in which DOX is encapsulated, with the aim of reducing the toxic effects against normal cells whilst increasing its therapeutic activity.

The choice of the polymer matrix used must fulfill several requirements such as biocompatibility, biodegradability, mechanical strength and ease of processing. The best known class of biodegradable materials for controlled release are the poly(lactide-*co*-glycolide)s (PLGAs). Various drug release profiles can be achieved by varying the molecular weight, copolymer ratio, drug loading, microparticle size and porosity, and the fabrication conditions. The

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fabrication technique chosen should be based on the nature of the polymer, the drug, its intended use and the duration of the therapy.

The present research report describes the in vitro release of DOX from PLGA-based microparticles fabricated using the spray-drying technique. This technique has been an important and widely used technique in the pharmaceutical, chemical and biochemical fields [6–8]. It is not only applicable to heat-resistant and heat-sensitive drugs, water-soluble and water-insoluble drugs, but can also be used to fabricate microparticles from hydrophilic and hydrophobic polymers. Furthermore, the polymeric microparticulate drug delivery systems produced using this technology have great potential in providing new routes of administration, such as oral dosage forms, targeting systems to organs and tissues and long acting parenteral biodegradable systems [9,10]. As DOX is highly water soluble, this technique, which does not involve the use of water, is the most suitable form of fabrication technique. In order to enhance the encapsulation efficiency (EE) of the drug in the polymer matrix, pluronic, a surfactant is also added during the fabrication process.

2. Materials and methods

2.1. Materials

Poly (L-lactic acid) (Cat. no. P1566 M_w 85,000–160,000), poly (DL-lactic-*co*-glycolic acid 65:35) (Cat. no. P2066 M_w 40,000–75,000), poly (DL-lactic-*co*-glycolic acid 85:15) (Cat. no. P1816 M.W.90,000–126,000), doxorubicin hydrochloride (Cat. no. D1515), and poly(ethylene glycol) (Cat. no. 20,245-2 Average M_w 8000) were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without modification. Pluronic P-105 (Cat. no. 9003-11-6) was purchased from BASF Corporation, polyethylene glycol phosphate buffer saline, (PBS) buffer used for in vitro release study, was bought from Sigma Aldrich containing 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.4. Ethylacetate (EA), methyl alcohol (MA), dichloromethane (DCM) (Cat. no. DR-0440) and acetonitrile (Cat. no. AS-1122) were purchased from Tedia Company Inc. (Fairfield, OH, USA). Propidium iodide (PI) (Cat. no. P1304MP) and coumarin6 (Product no. 8037L) were obtained from Molecular Probes Inc. (Eugene, OR, USA) and Polysciences Inc. (Warrington, PA, USA), respectively. All other materials and reagents used were of analytical grade.

2.2. Microparticles preparation

Various types of DOX-loaded PLGA-based microparticles were prepared using a laboratory scale Buchi mini spray dryer B-191 (Buchi Laboratory-Techniques, Switzerland) with a standard nozzle. The optimized operating condition chosen is given as: inlet temperature 70 °C, outlet temperature 50–55 °C, aspirator ratio 100%, compressed air flow 700 Nl/h and polymer solution feed rate 20%. These process parameters were kept constant for all samples fabricated. Pluronic was added as an additive to investigate its effect on the EE, size and size distribution, morphology, physicochemical properties and release kinetics. In the fabrication of single-walled microparticles, PLGA, DOX and pluronic were dissolved in an appropriate proportion of DCM or EA, and sonicated to obtain a homogeneous suspension. The solution or suspension system was then spray dried and stored in a vacuum dessicator at room temperature. In the fabrication of composite microparticles, DOX was first dissolved in methyl alcohol, then mixed with poly(L-lactic) acid (PLLA)/DCM solution and sonicated till a homogeneous solution was formed. Thereafter, the mixture was added dropwise to the PLGA/EA solution and sonicated before spray drying to obtain the microparticles.

2.3. Microparticles characterization

2.3.1. Encapsulation efficiency

EE is defined as the percentage of the actual mass of drug encapsulated in the polymeric carrier relative to the initial amount of drug loaded. In the determination of the EE, microparticles were accurately weighed and dissolved in a certain volume of DCM via sonication till complete solubility. The solution was then placed in a desiccator for complete evaporation of DCM, after which acetonitrile/sodium acetate (pH 4) solution was added and centrifuged. The drug content of each sample was then analyzed using reverse phase HPLC at the wavelength of 550 nm.

2.3.2. Size and size distribution

The particle size and its distribution were measured by a laser light scattering analyzer (90 Plus Particle Sizer, Brookhaven Instruments, USA). Deionized water was first filtered through a 0.2 μm filter prior to addition to a small quantity of sample in an optical cell. The optical cell was then sonicated in a water bath to disaggregate the particles and placed in the sample holder for measurement.

2.3.3. Microscopic studies

The scanning electron microscope (SEM, JSM-5600LV, JEOL, Tokyo, Japan) was used to study the shape and surface morphology of the particles. The specimens were coated with platinum using an Auto-fine Sputter Coater (JFC-1300, JEOL Co., Tokyo, Japan) for 40 s before analysis using the SEM.