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Controlled release of drug and better bioavailability using poly(lactic acid-*co*-glycolic acid) nanoparticles

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ABSTRACT

Tamoxifen (Tmx) embedded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles (PLGA-Tmx) is prepared to evaluate its better DNA cleavage potential, cytotoxicity using Dalton's lymphoma ascite (DLA) cells and MDA-MB231 breast cancer cells. PLGA-Tmx nanoparticles are prepared through emulsified nanoprecipitation technique with varying dimension of 17–30 nm by changing the concentrations of polymer, emulsifier and drug. Nanoparticles dimension are measured through electron and atomic force microscopy. Interactions between tamoxifen and PLGA are verified through spectroscopic and calorimetric methods. PLGA-Tmx shows excellent DNA cleavage potential as compared to pure Tmx raising better bioavailability. *In vitro* cytotoxicity studies indicate that PLGA-Tmx reduces DLA cells viability up to ~38% against ~15% in pure Tmx. Hoechst stain is used to detect apoptotic DLA cells through fluorescence imaging of nuclear fragmentation and condensation exhibiting significant increase of apoptosis (70%) in PLGA-Tmx vis-à-vis pure drug (58%). Enhanced DNA cleavage potential, nuclear fragmentation and condensation in apoptotic cells confirm greater bioavailability of PLGA-Tmx as compared to pure Tmx in terms of receptor mediated endocytosis. Hence, the sustained release kinetics of PLGA-Tmx nanoparticles shows much better anticancer efficacy through enhanced DNA cleavage potential and nuclear fragmentation and, thereby, reveal a novel vehicle for the treatment of cancer.

1. Introduction

Tamoxifen (Tmx) is an estrogen receptor modulator which is used chronically for the treatment of estrogen receptor positive breast cancer [1]. Chemotherapy is a very complicated and high risk procedure due to its severe side effects [2,3]. One of the major problems of cancer chemotherapy is to administer the required therapeutic concentration of drug at the tumor site for the desired period of time without causing undesirable effects on other organs [4,5]. Clinical application is limited for most anticancer drugs due to their adverse side effects. Hence, sustained drug delivery systems may help to overcome adverse side effects by controlling local concentration of the drug at the receptor site [6,7]. Amongst various biodegradable polymers, poly(D,L-lactic acid), poly(D,L-lactic-*co*-glycolic acid), and poly(ε-caprolactone) are being used extensively as polymeric nanoparticles for controlled and targeted delivery of drugs [8–10]. On the other hand, the antimicrobial activities against food pathogenic bacteria have been carried out using drugs embedded polymeric nanocomposite films [11,12]. Low toxicity, absorbable and degradation properties of these polyesters make possible to obtain biodegradable drug delivery systems. The properties of polyesters can be modified by altering molecular weight of polymer and copolymer composition of the polymeric matrices used for drug encapsulation [13–15].

Among various biodegradable polymers, poly(lactic-*co*-glycolic acid) (PLGA) is an approved biodegradable polymer with good biocompatibility and widely employed for loading and encapsulation of variety of anticancer drugs [16–18]. Controlled delivery of anticancer drugs by using PLGA nanoparticles have been reported by various groups [19]. Doxorubicin-loaded PLGA nanoparticles and its *in-vitro* evaluation have been reported by using MDA-MB-231 breast cancer cells [20]. Paclitaxel-loaded PEGylated PLGA-based nanoparticles and its *in-vitro* and *in-vivo* evaluation

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have been reported [21]. Pharmacodynamics of cisplatin-loaded PLGA nanoparticles has been carried out by using tumor-bearing mice [22]. Paclitaxel-loaded small PLGA nanoparticles have been developed, and studied their antiproliferative activity and molecular interactions on prostatic cancer cells [23] while regulated and sustained delivery is not reported in the literature so far.

In the present study, varying dimension of tamoxifen loaded PLGA nanoparticles (PLGA-Tmx) have been prepared using novel emulsified nanoprecipitation method. Physicochemical characterizations have been performed for the evaluation of particle size, interactions and encapsulation efficiency. The sustained release of drug has been demonstrated by regulating the size of PLGA-Tmx nanoparticles. The DNA cleavage potential was evaluated using PBR322 DNA. *In-vitro* anticancer activity was evaluated against Dalton's lymphoma ascite cells and MDA-MB231 breast cancer cells in terms of percentage cell viability, morphological cell density and apoptosis in terms of nuclear fragmentation and condensation.

2. Materials and methods

2.1. Materials

Poly(lactic-*co*-glycolic acid) (PLGA 50:50), poly(vinyl alcohol) (PVA) ($M_W \sim 30{,}000$), and tamoxifen were purchased from Sigma, USA. Dichloromethane (DCM) was obtained from Qualigens Fine chemical, India. Agarose, MTT and Hoechst 33258 dye were purchased from Sigma Aldrich, USA; PBR322 DNA from Merck, India; FBS from Invitrogen, USA; RPMI from Cell Clone; and L-glutamine, penicillin, streptomycin from Himedia, India. All chemicals were used of molecular biology and analytical grade.

2.2. Preparation and optimization of PLGA/PLGA-Tmx nanoparticles

PLGA nanoparticles were prepared by using emulsified (o/w) nanoprecipitation method. Optimization was made to obtain regulated size of PLGA nanoparticles with higher encapsulation efficiency. PLGA nanoparticles were prepared by using the modified nanoprecipitation technique [24–26]. Briefly, different concentrations of PLGA were prepared by dissolving in dichloromethane (DCM). The resulting organic solution was then added drop wise into aqueous phase containing PVA (emulsifier) under high speed stirring using vortex. The resulting suspension was then allowed to evaporate the organic solvent under mechanical stirring at room temperature (~25 °C). Nanoparticles were collected through ultracentrifugation (15,000*g*, 30 min, 10 °C) of the suspension and were washed with distilled water at least three times. Trapped DCM was removed from nanoparticles suspension using rotor evaporation technique followed by keeping the nanoparticles under reduced pressure at 37 °C. The final dried nanoparticles were stored at 4 °C until further use.

Different concentrations of Tmx and PLGA were prepared by dissolving in dichloromethane (DCM). PLGA-Tmx nanoparticles were synthesized by using emulsified nanoprecipitation technique to encapsulate tamoxifen (Tmx), a lipophilic anti-estrogenic drug, into PLGA matrix. Different concentrations of Tmx solution (5, 10, 15 and 20 wt.% with respect to PLGA) was prepared in DCM as solvent. Different concentrations of PLGA solutions (10, 20, 30 and 40 mg/ml) were prepared in the same solvent. Solutions of Tmx and PLGA were mixed and solubilized properly using vortex. For emulsification, the prepared organic solutions were dispersed in an aqueous phase containing PVA as emulsifier and stabilizer. The concentration of PVA was changed in the range of 1.0–3.0% (w/v) aqueous solution. The resulting PLGA solution including Tmx was added into PVA emulsifier for oil-in-water (o/w) emulsion. This emulsion was broken down into nanodroplets by applying homogenization and sonication using 33 kHz of frequency at ~25 °C. These nanodroplets form the nanoparticles upon evaporation of the highly volatile organic solvent (DCM) under reduced pressure at 37 °C. The preparation of emulsified nanoprecipitation temperature was varied from 15 to 35 °C for desired size of PLGA-Tmx nanoparticles with high entrapment efficiency.

2.3. Nanoparticles characterization

Transmission electron microscopy (TEM: Technai 12 G^2, FEI, The Netherland) operated at 100 kV, was performed to visualize the nature and the size distribution of PLGA/PLGA-Tmx nanoparticles. Average nanoparticles dimension was calculated from several individual particles in the cluster. Surface morphology of the nanoparticles was determined using scanning electron microscope (SEM), ZEISS, EVO-LS-10. Samples of nanosuspensions were prepared by placing one drop on a glass plate and were dried under reduced pressure. Nanoparticles were gold coated using sputtering apparatus before observation in SEM. Atomic force microscopy was performed using a *NT-MDT* multimode *AFM*, Russia, controlled by Solver scanning probe microscope controller. Tapping mode was used with the tip mounted on 100 μm long single beam cantilever with resonant frequency in the range of 240–255 kHz, and the corresponding spring constant of 11.5 N/m.

Thermal properties were measured using Mettler 832 DSC at the scan rate of 10° per minute to compare melting temperature and heat of fusion of PLGA and drug loaded nanoparticles (PLGA-Tmx). Around 4.0 mg of PLGA/PLGA-Tmx nanoparticles as sample size was used for DSC measurement. Melting point was taken from the peak position while the areas under the peaks provide the heat of fusion of crystal melting. Fourier transform infrared (FTIR) spectroscopy was performed (Nicolet-6700, USA) to understand the nature of interactions in drug loaded samples. Spectroscopic characterization of PLGA nanoparticles, Tmx and PLGA-Tmx was carried out by using UV–vis spectrophotometer (Jasco V 600, Japan). Requisite amount of pure PLGA nanoparticles, Tmx and PLGA-Tmx nanoparticles were used for the spectroscopic scan from 200 to 800 nm using appropriate reference. Zeta potentials of different formulations were analyzed by using Delsa, Beckman Coulter particle size analyzer. Nanosuspensions were diluted with ultrapure water for these analyses.

2.4. In-vitro *release*

In-vitro release characteristics of Tmx from PLGA-Tmx nanoparticles were carried out in phosphate buffer saline (PBS) at pH 7.4 and 37 °C. The released concentration of Tmx was determined at different time interval using UV–vis spectrophotometer after making suitable calibration curve at 275 nm [27]. The release profiles of Tmx were studied from PLGA-Tmx nanoparticles prepared from different concentrations such as 10, 20 and 30 mg/ml of PLGA. Korsmeyer–Peppas model was used to study the release kinetics of Tmx from PLGA-Tmx nanoparticles. The Eq. (1) describes the drug release from a polymeric system [28].

$$M_t/M_\infty = k \times t^n \quad (1)$$

where M_t/M_∞ is a fraction of drug released at time t as compared to release at infinite time, K is the release rate constant and 'n' is the release exponent. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release *vs.* log time.