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Preparation, characterization, and biodistribution of letrozole loaded PLGA nanoparticles in Ehrlich Ascites tumor bearing mice

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ABSTRACT

Letrozole (LTZ) incorporated PLGA nanoparticles were prepared by solvent displacement technique and characterized by transmission electron microscopy, poly-dispersity index and zeta potential measurement. Radiolabeling of free LTZ and LTZ-loaded PLGA NPs was performed with technetium-99m with high labeling efficiency. The labeled complex showed good in vitro stability as verified by DTPA challenge test. The labeled complexes also showed significant in vivo stability when incubated in rat serum for 24 h. Biodistribution studies of ^{99m}Tc-labeled complexes were performed after intravenous administration in normal mice and Ehrlich Ascites tumor bearing mice. Compared to free LTZ, LTZ-loaded PLGA NPs exhibited significantly lower uptake by the organs of RES. The tumor concentration of LTZ-loaded PLGA NPs was 4.65 times higher than that of free LTZ at 4 h post-injection. This study indicates the capability of PLGA nanopartcles in enhancing the tumor uptake of letrozole.

1. Introduction

In recent years nanotechnology, as applied to medicine, has brought significant advances in the diagnosis and treatment of diseases. Nanoparticles (NPs) are considered to be the best drug delivery system, have considerable potential for drug targeting and exhibit several advantages over conventional delivery systems (Hughes, 2005; Allemann et al., 1993). These are: high stability conferring long shelf lives, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and possibility of administration through variable routes (Allen et al., 1993). Nanoparticles can also be designed to allow controlled drug release from the matrix. All these properties enable improvement of drug bioavailability producing high level of pharmacological action and reduction of the dosing frequency (Soppimath et al., 2001). Drugs incorporated into nanoparticles can be targeted to a specific site of action with concomitant reduction in the quantity of drug required and dosage toxicity, enabling safe delivery of toxic therapeutic drugs as well as protection of non-target tissues and cells from severe side effects (Brannon-Peppas, 1995).

Breast cancer is the most common non-cutaneous cancer among women. It is the second leading cause of cancer-related deaths in the United States (Weir et al., 2003). The antiestrogen tamoxifen has long been used in the treatment of pre- and postmenopausal breast cancer (Vogel, 2001). However some breast cancer became resistant to tamoxifen, and in some cases the drug increases the risk of endometrial cancer (Brown, 2002). Nowadays the aromatase inhibitors, representing a new class of agents are considered as more effective than tamoxifen in the treatment of breast cancer (Miller, 1997). Letrozole (LTZ) is an oral non-steroidal aromatase inhibitor approved by United States FDA and has been introduced for the adjuvant treatment of hormonally responsive local or metastatic breast cancer (Cohen et al., 2002; Long et al., 2004). It decreases the amount of estrogen produced by the body and can slow or stop the growth of some breast tumors that need estrogen to grow. LTZ-loaded nanoparticulate formulation has been developed to facilitate controlled release and targeted delivery of drugs thereby enhancing its therapeutic efficacy.

Polymeric nanoparticles have recently been considered as promising carriers for anticancer agents (Brigger et al., 2002; Vauthier et al., 2003). Poly-D,L-lactic-co-glycolic acid (PLGA), a copolymer of lactic and glycolic acids, is an excellent synthetic non-toxic biodegradable copolymer (Jain, 2000). It has been widely applied to formulate hydrophobic as well as hydrophilic drugs into nanoparticulate delivery systems because of its excellent biocompatibility, biodegradability and bioresorbability. Incorporation

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of drugs into PLGA based nanoparticulate formulation results in significant change in tissue distribution profile, target specificity and pharmacokinetic behaviour (Yamaguchi and Anderson, 1993; Mainardes and Evangelista, 2005; Dillen et al., 2006). As these nanoparticles are small enough they are expected to circulate through capillaries, cross the highly permeable vasculature supplying blood to tumor (angiogenetic area), and enter tumor cells through endocytosis (Jin et al., 2008). All these may lead to improved therapeutic efficacy, better use of the drug, increased patient compliance and improved quality of life.

The objective of this study is to evaluate the biodistributive properties of free LTZ and LTZ-loaded PLGA NPs in mice bearing Ehlrich Ascites tumor and to investigate the capability of PLGA nanoparticles to enhance the tumor uptake of letrozole. LTZ-loaded PLGA NPs were prepared by solvent displacement technique using poloxamer-188 as stabilizer with slight modifications of the reported method (Mondal et al., 2008). Poloxamer-188 was used as surfactant to keep the precipitated particles suspended and discrete. Incorporation of poloxamer-188 in nanoparticulate formulation resulted in significant enhancement of the cytotoxic effect of the anticancer drug (Yan et al., 2010). The free drug (LTZ), and the LTZ-loaded PLGA NPs were radiolabeled with technetium-99m by direct radiolabeling approach using stannous chloride dihydrate as reductant. ^{99m}Tc-labeled LTZ and LTZ-loaded PLGA NPs were intravenously administered to normal and EA tumor bearing mice. Biodistributions in various organs, tumor uptake and retention were evaluated. The results are discussed in the following section.

2. Materials and methods

2.1. Chemicals

Poly-D,L-lactic-co-glycolic acid (PLGA), with a copolymer ratio of D,L-lactide to glycolide of 50:50 (Resomer 503H, average molecular weight 33,000 Da) and with inherent viscosity 0.32–0.44 dl/g, was purchased from Boehringer Ingelheim Co. (Ingelheim, Germany). Diethylene triamine pentaacetic acid (DTPA) and stannous chloride dihydrate were purchased from Sigma Chemical Co. (USA). Letrozole and poloxamer-188 (Lutrol 68) were gifted by Sun Pharmaceuticals Advanced Research Center (Baroda, Gujarat, India) and Ranbaxy Research Laboratory (Guragaon, India), respectively. $^{99}MoO_4^-$ was purchased from the Bhabha Atomic Research Centre (Mumbai) and $^{99m}TcO_4^-$ was obtained by 2-butanone extraction of a 5(N) NaOH solution of $^{99}MoO_4^-$. All other chemicals and solvents were of analytical grade and purchased from Merck India.

2.2. Preparation of letrozole loaded PLGA nanoparticles

The nanoparticles loaded with letrozole (LTZ) were prepared by solvent displacement technique as per the reported method (Mondal et al., 2008) with some alteration. Briefly a solution of 150 mg of PLGA (50:50) in 5 ml of acetone containing 50 mg of letrozole (drug polymer ratio 1:3) was added to an aqueous phase containing 0.5–1% (w/v) poloxamer-188 at a constant flow rate (0.3 ml/min) under mechanical stirring at 2000 rpm. The organic phase was evaporated at room temperature with constant stirring for 5 h. Finally the nanoparticles were isolated by centrifugation at 10,000 × g at 4 °C for 30 min and washed twice with double distilled water to remove free LTZ. The washings were discarded by centrifugation as described above. The suspension was then lyophilized (VIRTIS, Freeze Mobile, Model-6ES, Cambridge, USA) for 48 h using glucose and lactose (in the ratio of NPs:gluc:lact: 1:0.667:1.333 (w/w)) as lyoprotectant and cryoprotectant, respectively to obtain dry powdered nanoparticles.

2.3. Characterization of nanoparticles

LTZ-loaded PLGA NPs were characterized by different physicochemical methods. Both morphology and particle size distribution of nanoparticles were determined by transmission electron microscopy (TEM) on TECNAI SPIRIT model FE1 electron microscope (The Netherlands) as per reported protocol (Halder et al., 2008). Particle size distribution was also analysed by master sizer 2000 (Malvern instruments, UK). The polydispersity index of NPs was estimated by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern, UK) at a fixed angle of 90°. Samples were diluted with dust-free water to give the recommended scattering intensity. Analysis was carried at least for three times for each batch of sample and mean values were reported.

The particle charge of LTZ-loaded PLGA NPs was quantified by measurement of zeta potential by laser Doppler anmometry in Zetasizer Nano ZS (Malvern, UK). Samples were diluted with distilled water. The frequency shift or phase shift of an incident laser beam caused by these moving particles is measured as particle mobility, which is converted to zeta potential by the application of the Smoluchowski or Huckel theories.

The drug content of LTZ-loaded PLGA NPs was determined by centrifuging (25,000 rpm) the nano-dispersion at 4 °C for 1 h (Mondal et al., 2008). The residue was washed twice with distilled water and dried in vacuum. The yield was calculated based on the weight of the dry powder yield. The amount of entrapped letrozole in nano-dispersion was determined spectrophotometrically (UV-2450, Shimadzu, Japan) by shaking a known amount of nanoparticles in measured volume of methanol for 2 h and measuring the optical density (at 238 nm) of the entrapped drug present in the filtrate.

2.4. Radiolabeling of letrozole and letrozole loaded PLGA nanoparticles

LTZ and LTZ-loaded PLGA NPs were radiolabeled with technetium-99m (^{99m}Tc) by reduction with stannous chloride dihydrate as per the reported method (Richardson et al., 1977; Halder et al., 2008) with some modifications. Briefly aqueous $^{99m}TcO_4^-$ (74–148 MBq) was added either to aqueous solution of LTZ (1.8 mg/ml; pH adjusted to 4.0 with 0.05 N NaOH) or to drug loaded nanoparticles (equivalent to 1.8 mg/ml of letrozole; pH 4.0), this was followed by the addition of freshly prepared stannous chloride dihydrate solution (20 μl containing 20 μg of $SnCl_2$) to each and incubation for 15 min. Final pH of the reaction mixture was 3.75. The labeling efficiencies of LTZ and LTZ-loaded PLGA NPs were determined by ascending thin layer chromatography (TLC) using 2.5 cm × 10 cm silica gel strips (Merck Germany) as stationary phase and either acetone or ethanol: water (7:3) as mobile phase. The plates were developed after spotting the plates with test samples (2–3 μl) and quantitative analyses of the chromatograms were performed by cutting the strips into 1 cm pieces and counting them in a well-type gamma scintillation counter (Electronic Corporation of India Model LV4755, Hyderabad, India) at 140 keV.

2.5. Stability studies

In vitro stability of the ^{99m}Tc-labeled complexes of LTZ and LTZ-loaded PLGA NPs in 0.9% sodium chloride and serum was determined by ascending TLC technique. The labeled complex (0.5 ml) was mixed with 1.5 ml of normal saline or rat serum and incubated at 37 °C. The samples were withdrawn at regular intervals upto 24 h to monitor the stability by TLC.