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Note

In vitro and in vivo characterization of biodegradable enoxacin microspheres

Michael Abazinge[a], Tanise Jackson[b], Qing Yang[b], Godfried Owusu-Ababio[b,*]

[a]*Environmental Sciences Institute, Florida A&M University, Tallahassee, FL, USA*
[b]*College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL, USA*

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Abstract

The in vitro release and plasma concentration profiles of sustained release enoxacin microspheres intended for the treatment of bone and systemic infections due to sensitive strains of bacteria were investigated. Microspheres of enoxacin were prepared by using poly(glycolic acid-co-DL-lactic acid) (PLGA) by the emulsion solvent evaporation technique and characterized by in vitro release in an incubator, and in vivo release in the rat subcutaneous model. The microspheres were spherical in nature, and particle size range had a significant influence on the in vitro release. The enoxacin plasma concentration 2 h after the administration of treatments was two-fold higher in animals who received the free drug compared with those who received microspheres of size range 125–250 μm. The plasma of animals who received the free drug was depleted of enoxacin by the end of the first day. However, the plasma concentration of enoxacin in the animals who received microspheres was sustained above 0.5 μg/ml for about 8 days. The results show that biodegradable microspheres of enoxacin can be prepared which release the antibiotic in vivo for days following a subcutaneous administration. This should provide a means for the sustained treatment of infections due to sensitive strains of bacteria.

Keywords: Enoxacin; Solvent evaporation; Biodegradable polymer; Microspheres; Rat

1. Introduction

Enoxacin, a broad spectrum fluoroquinolone antibiotic, is active against both Gram-positive and Gram-negative organisms in vitro and in vivo [1,2]. However, enoxacin has a short pharmacokinetic half-life in humans and in a number of animals which has been quoted as 2–6 h [3–5]. Enoxacin concentration as high as 0.25 μg/ml is active against most Gram-negative bacteria [2], but rapid tissue distribution and a short pharmacokinetic half-life will require frequent administration of the antibiotic or a sustained or controlled release dosage form. This means that in diseases where bacterial biofilms can occur, such as those in bone infections, prosthetic devices, etc., the rapid decline in antibiotic concentration may result in resistance to treatment due to the occurrence of bacterial biofilm [6–8].

Polylactic and polyglycolic acids, including their copolymers, are the most widely used polymers for the preparation of biodegradable microparticles for the controlled release of biologically active agents because they degrade to monomers which are intermediates in mammalian energy metabolism [9]. These polymers are histocompatible and have been established by FDA licensure and years of use as absorbable sutures for medical application [10]. Furthermore, they avoid the need for surgical removal.

The aim of the present study was to design a sustained release dosage form of biodegradable microspheres of enoxacin for subcutaneous administration, and to compare the in vitro and in vivo release of enoxacin from the microspheres.

2. Experimental

2.1. Materials

Poly (glycolic acid-co-DL-lactic acid) (PLGA), composition 50:50 of viscosity grade 1.32 dl/g was supplied by Birmingham Polymers, Birmingham, AL. Polyvinyl alcohol (PVA) (MW 30 000–70 000) and enoxacin were obtained from Sigma Chemical Company, St. Louis, MO. Methylene chloride (HPLC grade), methanol (reagent grade), acetonitrile (HPLC grade), and acetone (regent grade) were

* Corresponding author. College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, USA. Tel.: +1-850-599-3111; fax: +1-850-599-8109.
E-mail address: gababio@famu.edu (G. Owusu-Ababio)

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supplied by Fisher Scientific Co., Norcross, GA. Distilled demineralized water was used. All materials were used as supplied.

2.2. Methods

2.2.1. Preparation of microspheres

Microspheres were prepared by the solvent evaporation procedure. Polyvinyl alcohol was dissolved in water to prepare 1% w/v solution. Five hundred milligrams of PLGA were dissolved in 10 ml of methylene chloride/acetone mixture (1:9), and 300 mg of enoxacin particles (less than 37 μm) were dispersed in the PLGA solution. Afterwards, the drug–polymer dispersion was added to 25 ml of the PVA solution which was being stirred at 800 rev./min with Lightnin Mixer (General Signal, NY). After 10 min the stir rate was reduced to 400 rev./min, and the organic solvent was evaporated overnight. The microspheres were collected by filtration, washed gently with methanol and water mixture, and dried in air for 2 days. All products were sieve sized using a combination of US standard sieve numbers 40, 60, and 120. Fractions collected between 40/60 (250–425 μm), and 60/120 (125–250 μm) were used for further studies.

2.2.2. Morphology of microspheres

The surface morphology, shape and size of enoxacin microspheres were obtained using a Jeol JSM-840 Scanning Electron Microscope (Jeol, Japan). Microspheres were coated with gold-palladium in a sputter coating apparatus before scanning electron microscopy (SEM) analysis.

2.2.3. Determination of encapsulation efficiency (EEF)

The amount of enoxacin in the microspheres was determined by dispersing 10 mg of the microspheres in 5 ml of methylene chloride which dissolved the polymer but not the drug. Enoxacin was extracted several times with an aqueous solution (pH 11) and analyzed spectrophotometrically. The amount of the drug in the microspheres was determined using an UV-1201 spectrophotometer (Schimadzu Scientific Instruments, Inc., MD) at 265 nm. The EEF was determined as the ratio of the amount analyzed to the initial amount of enoxacin added during preparation.

2.2.4. In vitro release of enoxacin from microspheres

Ten milligrams of free drug or an equivalent weight of drug-containing microspheres were used in all studies. The microspheres were dispersed in 200 ml phosphate buffer (pH 7.4) in closed containers in an incubator at 37°C, and agitated at 50 rev./ min. Samples of dissolved enoxacin were removed at specified time intervals for analysis and replaced with fresh buffer. Absorbance measurement was conducted by using an UV-1201 spectrophotometer at 265 nm. Concentrations of dissolved enoxacin were calculated from a standard curve.

2.2.5. In vivo evaluation of enoxacin from microspheres

Male Sprague–Dawley rats weighing 300–400 g were randomly assigned to control and experimental groups consisting of eight rats per group. On the first day of the experiment, each animal in groups 1, 2, and 3 received a single subcutaneous administration of 20 mg of free enoxacin, blank microspheres, and the equivalent of 20 mg of drug in microspheres, respectively. Microspheres of size range 125–250 μm or free drug was dispersed in 1 ml of sterile isotonic saline solution prior to administration. Blood samples of 0.3 ml were taken from the animals using the tail clip method. Sampling was repeated on days 2, 4, 8, and 12. The blood samples were immediately centrifuged after collection at 4000 × *g* for 15 min at 4°C and stored at −70°C until analysis. All of the animals were euthanized on day 12.

2.2.6. Analysis of enoxacin in plasma

A plasma sample of 0.1 ml was combined with 0.5 ml of acetonitrile to precipitate protein, vortexed for 20 s and then placed on ice for 10 min. The samples were centrifuged (15 600 × *g* for 10 min). The supernatant was then evaporated to dryness and reconstituted with 0.2 ml of distilled deionized water and analyzed by HPLC. Conditions of analysis included a C18 Nova-Pak column (Waters Corp., Milford, MA), a waters 515 HPLC pump connected to a Waters pump control module, a Waters 717 Plus Autosampler and a WatersTM Photodiode array detector. The HPLC system was operated by a Waters Millenum chromatography software version 2.15.01 run on a pentium computer. The mobile phase consisted of acetonitrile:33.5 mM phosphate buffer (20:80 v/v) adjusted to pH 3.0 with glacial

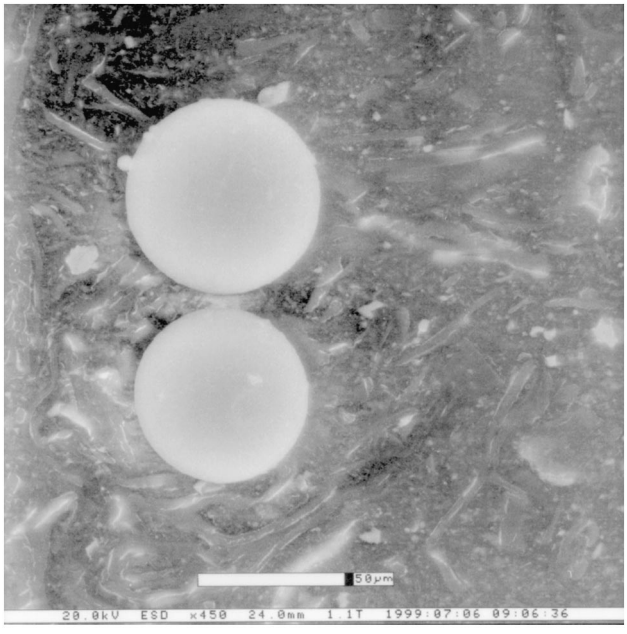

Fig. 1. Scanning electron micrograph showing the external characteristics of enoxacin mircospheres.

Table 1
Encapsulation efficiency for enoxacin microspheres of different sieve-size fraction within a batch

Particle size range	125–250 μm	250–425 μm
Encapsulation efficiency (%)	44.5 ± 1.3	61.1 ± 1.5

acetic acid. The retention time for Enoxacin was 2.7 min. The sensitivity of the assay was 0.1 μg/ml. The daily and day-to-day coefficients of variation were less than 6%.

3. Results and discussion

The scanning electron micrograph of a sample of microspheres collected in the sieve-size range 125–250 μm that deaggregated during preparation for scanning electron microscopy examination is shown in Fig. 1. It can be seen that the microspheres were spherical in nature. The encapsulation efficiency of microspheres of particle size range 125–250 μm was lower compared with microspheres of size range 250–425 μm (Table 1). The release of enoxacin from microspheres of size range 125–250 μm was complete in 14 days (Fig. 2). About 70% enoxacin was released in the first day from microspheres of size range 125–250 μm compared with 45% from microspheres of size range 250–425 μm. However, it can be seen from the release profiles in Fig. 2 that the rate of drug release after the third day from microspheres of size range 125–250 μm was similar to microspheres of size-range 250–425 μm. This is not unexpected since the particle size of a microparticle is known to influence the rate of drug release from microparticles [11]. Particle size influences the rate of drug release from microparticles predominantly by the total surface area exposed to the release medium.

The plasma concentration profiles of enoxacin released from enoxacin microspheres and enoxacin suspension are shown in Fig. 3. Analysis of samples taken 2 h post administration of treatment indicated that the enoxacin administered as a suspension attained a concentration of about 1 μg/ml, which was twice as high as that obtained from the microspheres. However, by the end of the first day, enoxacin was not detected in the plasma of the group of animals that received the enoxacin suspension. This is not surprising since the pharmacokinetic half-lives of enoxacin in mice, rats and humans have been quoted as 2–6 h [3–5]. In sharp contrast, the plasma concentration of enoxacin in the animals that received enoxacin microspheres rose gradually from 0.5 μg/ml at time 2 h to about 0.8 μg/ml by the end of the third day. However, enoxacin concentration in the plasma began to diminish when the microspheres were becoming depleted of the drug, and by the end of the eleventh day, enoxacin concentration had fallen to about 0.2 μg/ml.

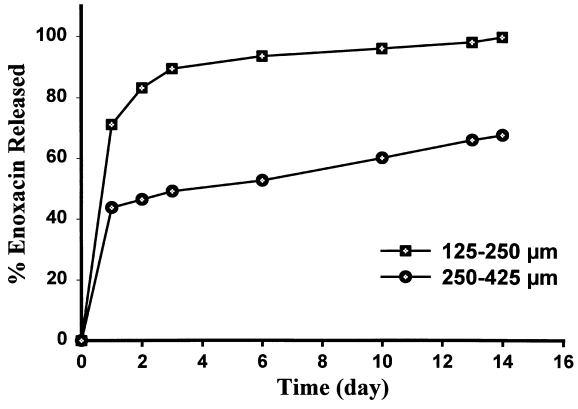

Fig. 2. The effect of particle size on the in vitro release of enoxacin from enoxacin microspheres prepared with poly(glycolic-co-DL-lactic acid) of viscosity grade 1.32 dl/g.

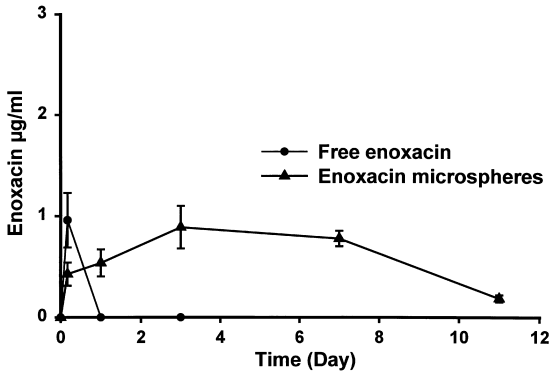

Fig. 3. The effect of delivery system on plasma concentration of enoxacin following the subcutaneous administration of 20 mg of free drug or the equivalent of 20 mg of the drug in microspheres.

The in vitro dissolution profile correlates with the in vivo plasma concentration profile (Figs. 2 and 3). The initial part of the in vitro release curve up to the third day from microspheres of size range 125–250 μm, correlates with the gradual rise in enoxacin concentration in the plasma to the maximum plasma concentration by the end of the third day. After the third day, enoxacin continued to be detected in the plasma until the 11th day, which correlates with over 96% enoxacin release in the in vitro release study.

It is apparent from these results that enoxacin microspheres can be prepared for subcutaneous administration which could provide sustained release of the drug in an animal model for several days using an erodible polymer. This contrasts with biodegradable gentamicin subcutaneous implants for the treatment of osteomyelitis which have to be administered under anesthesia, and gentamicin sulfate-loaded bone cement beads which have to implanted and removed under anesthesia [12,13].

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