Journal of Controlled Release 129 (2008) 192–199

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Synchronic release of two hormonal contraceptives for about one month from the PLGA microspheres: In vitro and in vivo studies

Yi Sun [a,b], JianCheng Wang [a], Xuan Zhang [a], ZhiJun Zhang [c], Yan Zheng [c], DaWei Chen [b], Qiang Zhang [a,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China
[b] Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China
[c] Beijing Zizhu Pharmaceutical Co., Ltd., China

ARTICLE INFO

Article history:
Received 16 November 2007
Accepted 25 April 2008
Available online 1 May 2008

Keywords:
Synchronic release
Hormonal contraceptives
Gestodene
Ethinyl estradiol
PLGA microspheres

ABSTRACT

A controlled drug release system based on the injectable PLGA microspheres loaded with gestodene and ethinyl estradiol was prepared and evaluated for the feasibility of monthly synchronic delivery of the two hormonal contraceptives. The scanning electron microscopy, light-scattering analyzer and gel permeation chromatography were used to study the morphology, particle size and molecular weight of the polymer microspheres, respectively. HPLC was utilized to determine the drug loading and the drug released, while a LC-MS-MS system was employed to analyze the plasma drug concentration. Result indicated that the PLGA particles obtained were spherical and appropriate in size. The formulation was stable during the test period. In vitro drug release from the microspheres for both drugs was sustained for about 30 days mostly by the diffusion mechanism. The plasma drug concentration-time profiles of the drug-loaded microspheres were relatively smooth after subcutaneous injection to rats for about 1-month, compared with that for drug suspension. In vitro and in vivo correlation was established. One of the most important facts is the synchronicity of the two contraceptives both in the release kinetics in vitro and the pharmacokinetic behaviors in vivo. Therefore, the synchronic delivery of two contraceptives is achieved for about 1 month by using the injectable PLGA-based microspheres.

1. Introduction

Hormonal contraceptives have played very important roles in family planning programs and have been used in clinical practice for more than 60 years. There has been a great increase in contraceptive use over the past 35 years in both developed and developing countries [1,2]. Ethinyl estradiol (EE) is a synthetic estrogen, while gestodene (GE) is a synthetic progestogen structurally related to levonorgestrel. They are commonly used as the estrogenic /progestogenic component in combination preparations [3,4].

The contraceptives can be formulated and administered by oral adminitration, injection, subdermal implants, transdermal patch or the vaginal ring [5]. However, oral route is the most commonly used since it is convenient and readily accepted by most patients. But oral contraceptives have high variations in blood concentration leading to blood-concentration-dependent side effects because of frequent administration [6–11]. And another drawback is that the reduction in efficacy due to the patient forgetting to take the medication, and resulting variation in drug levels in the blood descending to sub-therapeutic concentrations. Both side effects and forgetting to take the medication for the patient could be minimized by using a long-term controlled release system to ensure a longer period of steady blood drug concentration.

Currently, there are only a small number of commercially available products that utilize long-term controlled release technology. Norplant® is a silicone-based device for the delivery of levonorgestrel. However, the polymer used is not biodegradable, and the device has to be implanted and removed through surgical operation [12]. To overcome this problem, microspheres made of biodegradable polymer were developed for implantation under the skin without surgery [13].

Poly (D, L-lactide-*co*-glycolide) (PLGA), composed of poly (lactic acid) and poly (glycolic acid), is the most commonly used biodegradable copolymer. With the long history of safe human use in the form of surgical sutures, PLGA has been studied extensively as the injectable polymeric microspheres to prolong the release of therapeutic agents, such as antibiotics, anti-inflammatory drugs, anticancer drugs, steroids, peptides and proteins [14–21]. Some of the products are commercially available, for example, Lupron Depot®, with a controlled release of leuprolide acetate for 1 month [22–24] after subcutaneous administration.

There are only a few reports on the injectable PLGA microspheres containing contraceptives, and very few on the contraceptive combination. Recently, M. D. Dhanaraju et al. reported the preparation, morphology, drug loading, as well as the in vitro release of the PLGA microspheres loaded with levonorgestrel and EE [25].

* Corresponding author. Tel./fax: +86 10 82802791.
E-mail address: zqdodo@bjmu.edu.cn (Q. Zhang).

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doi:10.1016/j.jconrel.2008.04.022

In literature, there is little information related to PLGA microspheres loaded with the drug combination. Hence many important issues remain to be explored and resolved, such as the exact control of drug release, synchronic release of different drugs, simultaneous determination of two or more components, precise determination of trace quantity of drugs (like hormonal contraceptives) in vivo, establishment of the correlation between in vitro and in vivo studies, and so on.

Accordingly, in the present study, a newly developed controlled release system based on PLGA microspheres loaded with both GE and EE was prepared and evaluated. Besides the evaluation of physicochemical characteristics, the in vitro degradation of the PLGA microspheres was also investigated. In vitro release studies were conducted to ensure the control release and specifically, synchronic release of two components for about 1 month. An unreported analytical method based on LC-MS technique was also developed to simultaneously determine two trace components in vivo samples for pharmaceutical analysis [26,27]. Finally, the correlation between in vitro and in vivo release was established.

2. Materials and methods

2.1. Materials

Poly (D, l-lactide-co-glycolide) (PLGA, 50:50, Av. Mw 12,000, Inherent visc. 0.17 dL/g) was purchased from Absorbable Polymers International (USA). GE and EE (microcrystalline powder, 99% purity) were obtained from Beijing Zizhu Pharmaceutical Co., Ltd (Beijing, China). Methylnorethindrone (99.1% purity) was supplied by Shenyang Pharmaceutical University (Shenyang, China). Dansyl chloride (99.0% purity) was from Sigma-Aldrich (USA). Poly (vinyl alcohol) (PVA 05-88, Mw 27,000–32,000) and sodium carboxymethycellulose were the products of Beijing Organism Chemical Industry Co. Ltd. Methanol, Acetonitrile (Merck Chemical Co.) and tetrahydrofuran (THF) were of HPLC grade. Other chemicals were of reagent grade. All solutions were prepared with distilled water.

2.2. Preparation of PLGA microspheres

An oil-in-water (o/w) emulsion solvent extraction/evaporation method was used for the preparation of GE and EE microspheres [28]. Briefly, 1250 mg of PLGA, 25 mg of GE and 10 mg of EE were dissolved in 10 ml of dichloromethane. This organic phase was slowly added to 300 ml of 1% PVA aqueous solution. The mixture was homogenized at 12,000 rpm for 10 min. The obtained emulsion was then added to 800 ml of 1% (w/v) PVA aqueous solution and stirred at 250 rpm, 25 °C for 6 h. The resulting microspheres were washed three times with distilled water and lyophilized for 24 h (ALPHA2-4, CHRIST, Germany).

2.3. Characteristics of microspheres

2.3.1. Observation by scanning electron microscopy (SEM)

The morphology of the microspheres was examined using a scanning electron microscope (SEM, JSM-5600LV, JEOL, Tokyo, Japan); images of microspheres were also taken after the in vitro drug release tests. Samples were prepared by placing microspheres onto an aluminium specimen stub, then dried overnight followed by sputter coated with gold prior to imaging (IB-3 Ion Coater, EIKO, Japan). Coating was performed at 2 mA for 3 min.

2.3.2. Particle size analysis

A light-scattering particle size analyzer (BT-9300, BETTER, China) with a circulation disperser (BT-600, BETTER, China) was used to determine the size distribution of the prepared microspheres. The lyophilized particles were suspended by a large mount of distilled water and analyzed under continuous stirring. Particle size was expressed as volume mean diameter in micrometers (SEM, $n=3$) of three batches.

2.3.3. Molecular weight of PLGA by gel permeation chromatography

Gel permeation chromatography (GPC) was performed to determine the mean molecular weight of PLGA, using a Waters ® system (Waters, USA) which included a Waters 515 HPLC pump, three Waters Styragel columns (HT2, HT3, HT4), a temperature control module with column heater, a Waters 2410 Differential Refractometer, and a Waters 2487 Dual λ Absorbance Detector. The pump flow rate was set at 1.0 ml/min and the columns were held at a constant temperature of 35 °C. Microspheres (10 mg) were dissolved in 10 ml of THF and filtered (0.22 μm). Only glassware (vials and syringes) was utilized to minimize possible contamination from plastic materials. The polymer solution injection volume was 50 μl. The data collection and analysis were performed using Waters Millennium[32] software. Weight average molecular masses were calculated based on polystyrene standards. All the measurements were conducted in triplicate and the mean values and standard deviations were reported.

2.3.4. High performance liquid chromatography

The concentrations of GE and EE in the in vitro studies were determined using HPLC system (HP1100, Angilent, USA), consisted of a pump and a UV-Vis detector set at 273 nm. The analytical column was a DIAMONSIL® C18 (4.6 mm × 250 mm) (Dikma technologies, Beijing, China) and the column temperature was set as 30 °C. The mobile phase consisted of methanol and water at a ratio of 65:35 (v/v) and the flow rate was 1 ml/min. Aliquot of 50 μl clear supernatant was injected into the HPLC system. The peak areas of GE and EE were recorded and the concentrations were calculated from a standard curve. The retention time of GE and EE was 18 min and 21 min, respectively.

2.3.5. Drug loading efficiency

To determine loading percentage of GE and EE in the microspheres, 10 mg of the freeze-dried microspheres were dissolved in 1 ml of dichloromethane. The resulting solution was then diluted to 10 ml with HPLC mobile phase and vortex-mixed (WH-861 Vortex Shaker, China). One milliliter of the dispersion was centrifuged for 10 min at 10,000 rpm (TGL-16G centrifuge, Shanghai, China). The supernatant was injected into HPLC system to determine the concentration of GE and EE. The drug loading efficiency (LE) was calculated as follows:

$$LE = (\text{drug found in microspheres}/\ \text{drug added}) \times 100\%$$

All the measurements were conducted in triplicate and the mean values and standard deviations are reported.

2.3.6. In vitro drug release studies

Aliquot of 15 mg freeze-dried microspheres were suspended in 30 ml of 0.1 M PBS (pH 7.4) containing 0.05% Tween 80 and 0.02% sodium azide and stirred at 50 rpm in an air chamber thermostated at 37 ± 1 °C. At appropriate intervals, samples were collected and centrifuged at 3000 rpm for 5 min. Aliquot of 1 ml supernatant was taken and replaced with the equal value of fresh release medium. Then the supernatant was centrifuged at 10,000 rpm for 10 min. The amount of GE and EE in the collected supernatant was measured by the HPLC method (see Section 2.3.4). All the release tests were performed in triplicate.

2.3.7. In vitro degradation of the microspheres

Aliquot of 15 mg freeze-dried microspheres was dispersed in 30 ml of 0.1 M PBS (pH 7.4) containing 0.05% Tween 80 and 0.02% sodium azide. And the resulting suspension was dispersed in tubes and stirred at 50 rpm in an air chamber thermostated at 37 ± 1 °C. At the specific time point, the microspheres were collected by centrifugation (3000 rpm, 5 min), washed three times with double-distilled water and lyophilized. The samples were weighed and the weight reduction of the microspheres was evaluated using the following equation:

$$\text{Weight reduction} = M_2/M_1 \times 100\%$$