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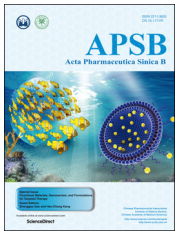

ORIGINAL ARTICLE

Preparation and evaluation of a phospholipid-based injectable gel for the long term delivery of leuprolide acetaterrh

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Abstract A phospholipid-based injectable gel was developed for the sustained delivery of leuprolide acetate (LA). The gel system was prepared using biocompatible materials (SPME), including soya phosphatidyl choline (SPC), medium chain triglyceride (MCT) and ethanol. The system displayed a sol state with low viscosity *in vitro* and underwent *in situ* gelation *in vivo* after subcutaneous injection. An *in vitro* release study was performed using a dialysis setup with different release media containing different percentages of ethanol. The stability of LA in the SPME system was investigated under different temperatures and in the presence of various antioxidants. *In vivo* studies in male rats were performed to elucidate the pharmacokinetic profiles and pharmacodynamic efficacy. A sustained release of LA for 28 days was observed without obvious initial burst *in vivo*. The pharmacodynamic study showed that once-a-month injection of LA-loaded SPME (SPME-LA) led to comparable suppression effects on the serum testosterone level as observed in LA solution except for the onset time. These findings demonstrate excellent potential for this novel SPME system as a sustained release delivery system for LA.

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Abbreviations: LA, leuprolide acetate; LHRH, luteinizing hormone release hormone; MCT, medium chain triglyceride; NMP, *N*-methyl-2-pyrrolidone; SPC, soya phosphatidyl choline; SPME, the injectable gel system (soya phosphatidyl choline, medium chain triglyceride and ethanol); SPME-LA, LA-loaded SPME; VPGs, vesicular phospholipid gels.

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1. Introduction

Prostate cancer is the most commonly diagnosed cancer and accounts for high mortality among men. Routine treatments for prostate cancer include surgical intervention, radiotherapy and chemotherapy. Since prostate cancer cells depend on androgens for proliferation and growth, androgen deprivation remains the most prevalent treatment for prostate cancer. This is especially the case for advanced prostate cancer[1]. Clinically, androgen deprivation therapy includes medical and surgical castration. Notably, the effects of medical castration are comparable to surgical castration with bilateral orchiectomy[2].

Regarding medical castration, luteinizing hormone release hormone (LHRH) agonist analogs are often used for the effective downregulation of LHRH receptors[3], thereby indirectly decreasing testosterone levels. Among various analogs, leuprolide acetate (LA), a potent LHRH agonist, is often used clinically for medical castration. As a water-soluble peptide, LA requires repeated injections to obtain desirable therapeutic effect due to extremely short half-lives *in vivo*[4]. The poor patient compliance resulting from repetitive injections greatly limits this medical use for LA. To solve this problem, various long-term sustained-release preparations, such as microspheres[5–7], implants[8] and *in situ* forming implants[9–11] have been prepared and studied. Among these, *in situ* forming implants based on phase separation show several advantages including ease of preparation and lack of required surgery. After being subcutaneously administered in liquid form, *in situ* forming implants gradually form a solid or semisolid drug delivery depot at the injection site by solvent exchange. Eligard[12], a commercial *in situ* forming implant of LA, shows sustained drug release over one-, three-, four- and six-month periods. Nevertheless, *in situ* forming implants are also associated with potential safety concerns for the presence of high organic solvents in the polymer matrix, such as *N*-methyl-2-pyrrolidone (NMP, about 55%, *w/w*)[13]. Moreover, the high initial burst due to fast phase-transition of the implants *in vivo* remains unresolved[14].

Our group recently developed a novel injectable *in situ* forming gel delivery system. The gel system, herein named SPME, was prepared by mixing soya phosphatidyl choline (SPC), medium chain triglyceride (MCT) and ethanol. SPME has several advantages over poly(lactide-*co*-glycolide) (PLGA)-based *in situ* forming implants, namely minimal amounts of organic solvent, excellent biocompatibility, and significantly less burst effects. Unlike most polymeric *in situ* forming implants, the novel SPME system is based on the phase separation of phospholipids *in vivo*. As the main component of cell membrane, phospholipids are biologically compatible, and soluble in ethanol, yet display poor solubility in water. When administrated in the liquid form, the SPME system undergoes phase separation, thus resulting in the formation of a solid or semisolid gel at the injection site due to the *in situ* water–ethanol exchange between the surrounding body tissues and the SPME system. Phospholipid-based *in situ* forming gel drug delivery systems have been successfully applied to the long term delivery of various drugs, such as doxorubicin[15], bromotetrandrine[16] and exenatide[17]. Previous studies reported that when loaded with octreotide, the gel system showed extended release for 30 days *in vivo*[18].

The present study aimed to investigate the application of the SPME system as a long term drug delivery platform for LA. The *in vitro* and *in vivo* release profiles of LA from the SPME system were investigated systematically. Next, the stability of LA in the SPME system was investigated under different temperatures and in the presence of various antioxidants. Finally, *in vivo* studies in male rats were performed to elucidate the pharmacokinetic profiles and pharmacodynamic efficacy of this preparation.

2. Materials and methods

2.1. Materials

Soya phosphatidyl choline (SPC, Lipoid S100) was obtained from Lipoid (Germany, 579010-1140034-04/902). Medium chain triglyceride (MCT) was obtained from Beiya Medical Oil Co., Ltd. (Tieling, China, y110501-3-01). Ethanol was provided by Tianhua (Chengdu, China). Leuprolide acetate (LA) was supplied by Kaijie (Chengdu, China). (±)-α-Tocopherol was obtained from Sigma–Aldrich (St Louis, MO, USA). Glycine was provided by Kemiou (Tianjin, China). All the other chemicals and reagents were of analytical grade.

2.2. Animals

Male Sprague–Dawley (SD) rats (8 weeks old, 300 ± 20 g) were obtained from Experimental Animal Center of Sichuan University (Chengdu, China). During the study, the rats were given free access to food and water *ad libitum*. All animal experiments were performed according to China's Animal Welfare Legislation and the Institutional Animal Care and Use Guidelines of Sichuan University.

2.3. Preparation of SPME-LA and evaluation of viscosity

LA-loaded SPME (SPME-LA) was prepared by mixing SPC, MCT, and 90% (*v/v*) ethanol (70:15:15, *w/w/w*) and stirred for 1 h. For viscosity measurement, dialysis method was applied to simulate the phase separation process. A volume of 20 mL SPME-LA was put into a dialysis bag and then incubated in 4 L of 0.1 mol/L PBS (pH 7.4) under shaking. The Brookfield DV-C rotational viscometer was used to measure the viscosities of SPME-LA before (sol) and after phase separation (gel).

2.4. In vitro release experiment

LA solution (300 μL, 3.75 mg/mL) or SPME-LA (300 μL, equivalent to 3.75 mg/mL LA) was added into dialysis bags and the dialysis bags were placed in 4 mL of different release media containing 0.1 mol/L PBS (pH 7.4) with varying percentages of ethanol (0, 10%, 20% or 30%, *v/v*). The release setup was maintained under shaking at 37 °C. At given time intervals, the entire media was removed and replaced with prewarmed fresh media. The concentration of LA in the release media was analyzed by high performance liquid chromatography (HPLC, Agilent Technologies 1200 series) using UV detection at 220 nm, a Kromasil C18 column (150 mm × 4.6 mm, 5 μm) at 30 °C. The mobile phase consisted of 0.1% trifuroacetic acid and acetonitrile (68:32, *v/v*), and flowed through the column at the rate of 1.0 mL/min. The cumulative released amount of LA was calculated according to the following equation (1) [19]:

$$\text{Cumulative amount released } (\%) = \left(\sum_{t=0}^{t} \frac{M_t}{M_{\text{theoretical}}} \right) \times 100 \quad (1)$$

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