International Journal of Pharmaceutics 427 (2012) 410–416

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Preparation, characterization and pharmacokinetic studies of tacrolimus-dimethyl-β-cyclodextrin inclusion complex-loaded albumin nanoparticles

Shanshan Gao[b], Jun Sun[b], Dongjun Fu[b], Hongli Zhao[a], Minbo Lan[a], Feng Gao[a,b,c,*]

[a] Shanghai Key Laboratory of Functional Materials Chemistry, East China University of Science and Technology, Shanghai 200237, PR China
[b] Department of Pharmaceutics, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, PR China
[c] Shanghai Key Laboratory of New Drug Design, East China University of Science and Technology, Shanghai 200237, PR China

ARTICLE INFO

Article history:
Received 15 November 2011
Received in revised form
30 December 2011
Accepted 24 January 2012
Available online 2 February 2012

Keywords:
FK506/DM-β-CD inclusion complex
BSA nanoparticle
Sustained-release

ABSTRACT

The purpose of the study is to develop a new formulation for clinically used anti-cancer agent tacrolimus (FK506) to minimize the severe side effects. Toward this end, a new formulation method has been developed by complexation of FK506 with an hydrophilic cyclodextrin derivative, heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) using ultrasonic means. The resulting complex displays dramatically enhanced solubility of FK506. Then bovine serum albumin (BSA) nanoparticles were prepared directly from the preformed FK506/DM-β-CD inclusion complex by the desolvation-chemical crosslinking method, with the size of 148.4–262.9 nm. Stable colloidal dispersions of the nanoparticles were formed with zeta potentials of the range of −24.9 to −38.4 mV. The entrapment efficiency of FK506 was increased as high as 1.57-fold. Moreover, notably FK506 was released from the nanoparticles in a sustained manner. As demonstrated, pharmacokinetic studies reveal that, as compared with FK506-loaded BSA nanoparticles, the FK506/DM-β-CD inclusion complex-loaded BSA nanoparticles have significant increase at T_{max}, $t_{1/2}$, *MRT* and decrease at C_{max}. In summary, these results suggest that the drug/DM-β-CD inclusion complex-loaded BSA nanoparticles display significantly improved delivery efficiency for poorly soluble FK506 or its derivatives.

1. Introduction

Tacrolimus (FK506, molecular weight of 822.05, water solubility of 1.3 μg/ml), a hydrophobic macrolide lactones natural product isolated from by *Streptomyces tsukubaensis*, exerts potent immunosuppressive effects and has been in clinical use as prophylaxis against organ rejection after liver and renal transplantation (Hidetoshi et al., 2001). Recently, it has been reported that FK506 can be widely distributed in the body with a high degree binding of red blood cells and plasma proteins. However, the distribution is significantly affected by individual differences, and the administration routes. For example, the gastrointestinal tract has a narrow therapeutic window due to low bioavailability (Taher et al., 2009), and side effects. In addition, FK506 is known to exhibit low oral bioavailability and a wide range of variability in absorption, ranging from 4 to 89% in kidney and liver transplant recipients (Venkataramanan et al., 1995). For the intravenous administration, because of its low solubility, some solubilizer or injection oil was used, thus inducing greater toxicity.

β-Cyclodextrin (β-CD) and its derivatives, as distinct solubilizers, have received considerable attention, giving prominence to their low biotoxicity and high biocompatibility. As attractive materials for drug inclusion, β-CD can be further chemically modified to improve its physicochemical properties (Hassan and Asghar, 2009; Song et al., 2009). A number of studies have shown that adding β-CD can improve the loading efficiency of nanoparticles and slow down the release of drugs (Alexander and Maria, 2007; Boudad et al., 2001; Maestrelli et al., 2006). Ferreira and collaborators prepared inclusion complexes with hydroxypropyl-β-cyclodextrin and the aqueous solubility of the drug increased linearly with the concentration of the cyclodextrins (Denise et al., 2004). The cavity depth and surface activity of 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD), a derivative of β-CD, improved significantly, as well as the solubility increased as much as by 25 times (Gamal et al., 1986). Various β-CD derivatives have been evaluated to probe their enhancing effect on solubility and stability of FK506 in rats. It was found that DM-β-CD had a dramatic improvement on solubilization and stabilization of FK506 (Hidetoshi et al., 2001).

Modern nanotechnology is considered as an emerging and converging technology (Roco, 2008) and that is said to be one of the

* Corresponding author at: Department of Pharmaceutics, School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, PR China. Tel.: +86 21 6425 2449; fax: +86 21 6425 8277.
E-mail address: fgao@ecust.edu.cn (F. Gao).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2012.01.054

key technologies of the 21st century. Nanotechnology is widely seen as having huge potential to bring benefits to many areas of research and application. Studies have shown that albumin nanoparticle has a higher capacity of loading hydrophilic drugs, better performance of controlled release and more stability of storage (MacAdam et al., 1997; Orapin et al., 1993). Furthermore, albumin offers several notable characteristics, including safety, non-toxic, non-immunogenicity, biodegradability and good biocompatibility. Therefore it serves an attractive drug delivery system. After release of drugs, albumin nanoparticles can be absorbed by body through the metabolic decomposition without producing harmful residual substances (Langer et al., 2003; Yun et al., 2007). In addition, the albumin molecule has many functional groups, thus allowing for convenient functionalizations for various purposes. General methods for preparation of albumin nanoparticles include ultrasonic emulsification, desolvation method, pH coagulation, salting, etc. (Langer et al., 2003; Muller et al., 1996; Merodio et al., 2001; Weber et al., 2000). For the desolvation method, several factors can affect albumin nanoparticles such as drugs, the dosage of albumin and the preparation of albumin nanoparticles including dehydrolyzing agent, cross linking agent, pH, cross linking time and stirring speed (Guilin et al., 2008; Hyuncheol et al., 2009; Vogel et al., 2002). With the carrier of albumin, albumin nanoparticles are solid sphere obtained by curing and separation, which can encapsulate and adsorb different kinds of drugs such as polypeptides, vaccines and gene to be used in varied disease. Controlled the nanosize of albumin nanoparticles can not only reduce toxicity but achieve certain sustained effect when used in intravenous. Currently, the research of FK506-loaded nanoparticles has mainly focused on the use of polylactic acid and acrylic acid as the carrier material (Nakaoka et al., 1995; Nakase et al., 2000). The study on the FK506/DM-β-CD inclusion complex-loaded albumin nanoparticles has not been reported.

The purpose of the present study is to develop a novel nano-drug delivery system (NDDS), drug/DM-β-CD inclusion complex-loaded BSA nanoparticles. Firstly, FK506 was complexed to a hydrophilic cyclodextrin derivative via the formation of inclusion complex of the drug with DM-β-CD by an ultrasonic method. Then, FK506/DM-β-CD inclusion complex-loaded bovine serum albumin (BSA) nanoparticles were prepared by desolvation-chemical crosslinking method. The physicochemical characteristics were determined (i.e. entrapment efficiency, loading efficiency, in vitro release, size distribution of the developed nanoparticles). In addition, pharmacokinetic parameters of these nanoparticles were investigated in rats.

2. Materials and methods

2.1. Chemicals and reagents

FK506 (purity > 99.1%) was purchased from Qiao Chemical Co. Ltd. (Shanghai, China). DM-β-CD (purity > 99.0%) was purchased from Kaiyang Biotech Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA, purity 96–99%) was purchased from Yuanju Bio-tech Co. Ltd. (Shanghai, China). Glutaraldehyde was obtained from Chinese Medicine Group Shanghai Chemical Reagent Company (Shanghai, China). Male Wistar rats used in the experiments were supplied by the Department of Laboratory Animal Science, Fudan University, treated according to the protocols evaluated and approved by the Ethical Committee of the University. All other reagents used in this study were high performance liquid chromatography (HPLC) or reagent grade.

2.2. Determination of FK506 by HPLC in vitro and in vivo

FK506 was determined using a modified reverse-phase HPLC system (Lee et al., 1995). This system consisted of two model LC-10ADvp pumps, an SPD-10AVP diode-array UV–Vis detector and an SCL-10AVP system controller. Chromatographic separation was achieved on a Platisil ODS-C_{18} column (4.6 mm × 250 mm, 5 μm, Dikma, Beijing, China). The mobile phase was consisted of acetonitrile–water (60:40, v/v) and adjusted to pH 2.7 with phosphoric acid, which delivered at an isocratic flow rate of 1.0 ml/min at the room temperature. The detection wavelength was set at optical density of 215 nm. The injection volume was 20 μl and the retention time was 9.8 min. Standard curve produced by the FK506 assay method appeared in a good linear correlation in the solution concentration range. The usefulness of the assay was confirmed by the analysis of plasma samples in rats.

2.3. Preparation of empty BSA nanoparticles

Empty BSA nanoparticles were prepared using a previously described desolvation technique (Muller et al., 1996). In principle, BSA was dissolved at concentrations of 10, 25, 50, 75.100 and 150 mg/ml in 2 ml of 10 mM sodium chloride and the pH of the solution was adjusted titrated to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, respectively. The resulting solutions were filtered through a 0.22-μm filter membrane. Aliquots (2 ml) of the BSA solution were transformed into nanoparticles by the continuous addition of 4–8 ml ethanol under constant stirring (600 rpm) at room temperature. The ethanol addition was performed using a constant flow pump which enabled nanoparticle preparation at a define rate of 1.0 ml/min. Following the desolvation process the particles were stabilized by the addition of an aqueous (150 μl) 4.17% glutaraldehyde solution. The crosslinking process was performed under stirring of the suspension over a time period of 18 h at room temperature. The resulting nanoparticles were purified repeated centrifugation at 12,000 rpm for 20 min, and redispersed in water by ultrasonication in order to eliminate excipients such as ethanol and glutaraldehyde. Dry nanoparticles were obtained after freeze drying with 3% mannitol as lyophilized protection agent.

2.4. Degradation of BSA nanoparticles

Nanoparticle degradation was determined in the absence and presence of trypsin. BSA nanoparticles were prepared as outline above except for the extent of particle stabilization. A known amount of the freeze-dried BSA nanoparticles was placed in phosphate buffer solution (PBS, pH 6.8) with and without 1% trypsin and incubated at 37 °C water-bath. The turbidity of the nanoparticle suspensions was measured photometrically at a wavelength of 565 nm by UV spectrophotometer (T6-1650F, Beijing, China). The BSA nanoparticle preparation was performed in three independent samples. The analytical results were given as mean value and standard deviation of these samples.

$$\text{degradation rate}\,(\%) = \frac{\text{initial concentration of the nanoparticles} - t\ \text{time concentration of the nanoparticles}}{\text{initial concentration of the nanoparticles}} \times 100 \quad (1)$$

2.5. Swelling of BSA nanoparticles

The swelling degrees of the BSA nanoparticles in demineralized water were determined in 24 h. A known amount of BSA nanoparticles was dispersed in water and the shaking rate was 150 rpm. After incubation at 37 °C at specified time, the suspension was