International Journal of Biological Macromolecules 109 (2018) 544–550

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Formulation and *in vivo* pharmacokinetic evaluation of ethyl cellulose-coated sustained release multiple-unit system of tacrolimus

Taek Hwan Shin [a], Myoung Jin Ho [b], Sung Rae Kim [a], Sung Hyun Im [b], Chang Hyun Kim [a], Sangkil Lee [c], Myung Joo Kang [b,*], Young Wook Choi [a,*]

[a] College of Pharmacy, Chung-Ang University, 221 Heuksuk-dong, Dongjak-gu, Seoul 156-756, Republic of Korea
[b] College of Pharmacy, Dankook University, 119 Dandae-ro, Dongnam-gu, Cheonan, Chungnam 330-714, Republic of Korea
[c] College of Pharmacy, Keimyung University, Daegu 704-701, Republic of Korea

ARTICLE INFO

Article history:
Received 14 November 2017
Received in revised form
13 December 2017
Accepted 20 December 2017
Available online 23 December 2017

Keywords:
Tacrolimus
Sustained release
Ethyl cellulose
Hypromellose
Reservoir-type pellets
Pharmacokinetics

ABSTRACT

A novel once-a-day sustained-release (SR) system of tacrolimus (FK506), a poorly water-soluble immunosuppressive agent, was designed employing ethyl cellulose (EC) polymer as release retardant. Drug (5 mg) was layered onto sugar spheres (518.3 mg) with hypromellose (5 mg), to transform the drug from a crystalline to an amorphous form. Subsequently, the drug-layered pellets were recoated with EC polymer (0.5-1.5 mg) using a fluid bed granulator. Drug release from the reservoir-type pellets was markedly impeded by the outer EC-based coating layer (EC 1 mg), displaying about 60% of drug release after 8 h, regardless of the acidity of the media. In an *in vivo* pharmacokinetic study in fasted Cynomolgus monkeys, the drug level in blood was gradually increased over 4.7 h and high drug concentration was maintained until 24 h, with an elimination half-life of 16.6 h. There were no statistical differences between the novel SR pellets and the recently marketed SR capsule (Advagraf®, Astellas Pharma, Japan) in terms of maximum blood concentration, area under the curve, and half-life values, in both fasted and fed states. Therefore, the novel EC-coated pellets are expected to be bioequivalent to the commercial SR capsule, providing a once-daily dosing regimen in patients with allogenic rejection.

1. Introduction

Tacrolimus (FK506) is an immunosuppressive agent commonly prescribed to reduce the rate of allograft rejection after hepatic and renal transplantation procedures [1,2]. The macrolide compound inhibits calcineurin by forming a complex with the FK506-binding protein, thereby impairing the transcription of interleukin-2 and other cytokines in T lymphocytes [3]. However, the oral therapy of FK506 has been hampered by its low and erratic intestinal absorption [4]. In order to enhance dissolution rate and improve oral absorption, several pharmaceutical approaches such as oily solution [5], solid dispersion (SD) [6], inclusion complex [7], and self-emulsifying drug delivery system [8] have been attempted. Among them, SD system with cellulose derivatives was commercialized (Prograf®, Astellas Pharma, Tokyo, Japan). However, to maintain the therapeutically effective concentration in blood, the current immediate release (IR) dosage form should be administered twice-a-day [9]. When considering the long-term use of the immunosuppressive agent after organ transplantation, the development of a once-a-day sustained release (SR) dosage form is highly desirable in terms of improving patient compliance and overall therapeutic outcomes.

Multiple-unit delivery systems consisting of individual pellet-like subunits have gained increasing attention as a platform to design SR dosages [10–12]. Even distribution of active compounds over multiple pellets reduces the variability in release profiles, providing less inter- and intra-subject variability in intestinal absorption compared to single unit dosage forms, such as coated tablets. Moreover, multiple-unit systems cause less mucosal irritation because individual sub-units are spread more broadly throughout the gastrointestinal tract [13–15]. In particular, reservoir-type SR pellets that have a separate release-controlling polymeric layered onto the drug-layered beads are favored to prevent drug-polymer interaction, fast initial release and/or incomplete release, which are potentially produced in matrix systems [16]. In the reservoir-type SR system, drugs and core materials are initially dissolved in aqueous medium taken up into the inner cores, and are subsequently released across the outer polymeric membrane into the gastrointestinal fluids [17,18]. When the

* Corresponding authors.
E-mail addresses: kangmj@dankook.ac.kr (M.J. Kang), ywchoi@cau.ac.kr (Y.W. Choi).

https://doi.org/10.1016/j.ijbiomac.2017.12.111
0141-8130/

concentration of the active compound is kept in the inner core compartment, a stationary concentration gradient across the polymeric membrane offers diffusion through this rate-controlling membrane at a constant rate.

Ethyl cellulose (EC) is a water-insoluble and pH-independent release polymer obtained by etherifying hydroxyl groups of the cellulose backbone with ethyl groups [19]. EC's water-insoluble and pH-independent properties have been extensively exploited for moisture protection, taste masking and controlled drug release from the oral dosage forms, for pharmaceutical applications [20,21]. EC polymer is suitable to prolong and/or modify the release of numerous drugs from solid dosage forms, including pseudoephedrine hydrochloride, acetaminophen and ibuprofen [22–24]. Drug release from the EC-coated pellets, which is mainly driven by osmotic pressure with a minor contribution by diffusion through aqueous pores, can be effectively controlled by adjusting the coating thickness of the cellulose derivative onto the pellets [17].

The aim of the present study was to construct a reservoir-type SR system of FK506, with the goal of a once-daily dosing regimen and improved patient compliance. FK506, which is poorly water-soluble, was primarily layered on sugar-based beads with hypromellose (HPMC). The HPMC-based solid dispersion (SD) onto the bead increased the thermodynamic solubility of the calcineurin inhibitor by altering the drug from a crystalline to an amorphous form, and by hindering drug recrystallization upon contact with the aqueous medium. Enhancement of the drug solubility in the inner compartment might support the effective control of drug release by the outer rate-controlling membrane onto the pellets. Drug-layered beads were subsequently coated with EC in a fluid bed granulator to retard release. The *in vitro* drug release profiles of the SR pellets coated with different amounts of the cellulose derivative were evaluated in various aqueous media. Pharmacokinetic profiles of the reservoir-type SR pellets was comparatively evaluated with the commercial SR product (Advagraf®, Astellas Pharma, US), a recently approved matrix-type SR formula, in Cynomolgus monkeys under fasted and fed states.

2. Experimental

2.1. Materials

Fk506 monohydrate was provided from Chongkundang Bio (Seoul, Korea). Sugar sphere was purchased from Pharma Line Inc. (Suwon-si, Gyeonggi-do, Korea). Hypromellose 2910 (HPMC, cps) and hydroxypropyl cellulose (HPC) were obtained from Shin-Etsu Chemical (Tokyo, Japan). Croscarmellose sodium was acquired from DMV-fonterra Excipients GmbH (Goch, Germany). Lactose hydrate and EC (Ethocel™, 7 cps) were obtained from Whawon Pharm. (Hwaseong-si, Gyeonggi-do, Korea). Polyethylene glycol 6000 (PEG 6000) was provided from Hwail Pharm. (Seongnam-si, Gyeonggi-do, Korea). All organic solvents including acetone, ethanol and acetonitrile were high-pressure liquid chromatography (HPLC) grade. All other chemicals were of reagent grade and were used with no further purification.

2.2. Drug layering process onto the beads

FK506-layered pellets (named IR pellets) were prepared using a fluid bed granulator. The composition of the IR pellets of the immunosuppressive agent is presented in Table 1. Drug powder (5.0% w/w) and hypromellose 2910 (5.0% w/w) were dissolved in a mixture of ethanol and methylene chloride (3:1 v/v). Lactose and croscarmellos sodium were homogeneously dispersed in the coating solution using a magnetic stirrer. The solution was introduced to the fluid bed granulator to allow coating of the drug onto the sugar sphere. The process parameters for the drug layering were: batch size, 400 g; inlet air temperature, 50–60 °C; product temperature, 40–50 °C; plasticizer feed rate, 40 g/min; and spray air pressure, 2 bar.

Drug crystallinity in the drug-layered pellets was assessed using X-ray diffractometry (XRD) and differential scanning calorimetry (DSC). To analyze the X-ray diffraction pattern, monochromatic Cu Kα-radiation (λ = 1.5418 Å) at a current of 30 mA and 40 kV voltage was used for drug-layered pellets, drug powder and blank pellets. The diffraction pattern over a 2θ range of 3–40° was obtained using a step size of 0.02° at a scan speed of 1 s per step (X'Pert Prompt PANalytical Co., Lelyweg, Netherlands). The thermal properties of the drug-loaded coating layer were analyzed using a DSC unit (DSC 50, Shimadzu Scientific Instruments, Kyoto, Japan). About 2 mg of sample was enclosed in a Tzero pan and lid, and subjected to heating at an elevating rate of 10 °C/minute over the range 45–300 °C under nitrogen gas purging. The flow rate of gas purging was set to 20 ml/minute.

2.3. EC coating onto drug-layered beads

The outer coating solution was prepared by dissolving the EC polymer (20% w/v), HPC polymer and PEG 6000 and subsequently suspending talc in ethanol anhydrous (Table 1). The resultant coating suspension was fed via a peristalic pump (Petro Gas Ausrüstungen GmbH, Berlin, Germany) and sprayed through a nozzle onto the drug-layered pellets (IR pellets). The coated pellets remained in the cyclic bed for 5 min. The parameters for coating processing were: batch size, 400 g; inlet air temperature, 50–60 °C; product temperature, 40–50 °C; plasticizer feed rate, 40 g/min; and spray air pressure, 2 bar.

2.4. In vitro *release test*

Release profiles of FK506 from the drug powder, drug-layered pellets, EC-loaded SR pellets, and the marketed SR product were evaluated according to US Pharmacopeia Apparatus 2 (paddle method; SR8 Plus dissolution apparatus, Hanson Inc, Chatsworth, CA, USA). Prior to the release test, drug powder, drug powder and pellets containing 5 mg of FK506 were filled into hard gelatin capsules. Each sample was placed in the 900 ml of dissolution medium (pH 1.2, pH 4.0, pH 6.8, and distilled water) and stirred at 50 rpm. The temperature of the media was kept at 37 °C ± 0.5 °C. At predetermined times aliquots (about 5 ml) were withdrawn and filtered using a 0.45 μm glass membrane syringe filter. An equal volume of the test media was refilled into the vessel. Samples were appropriately diluted and were analyzed using HPLC as described below.

2.5. HPLC analysis of FK506

The drug concentration in the samples was quantitatively analyzed using an Alliance 2695 HPLC system (Waters Corporation, Milford, MA, USA) equipped with a UV detector. The mobile phase consisted of acetonitrile, *tert*-butyl methyl ether and 6 mM phosphate solution in a volume ratio of 450:50:500, with a flow rate of 1.0 ml/min. One hundred microliters of sample was injected into the C18 column (5 μm, 4.6 mm × 150 mm, Waters Corporation) maintained at 60 °C. The column eluent was monitored at wavelength of 205 nm.

2.6. In vivo *pharmacokinetic studies in monkeys*

2.6.1. Animals and drug dosing

A single-dose, randomized, crossover pharmacokinetic study of the novel SR pellets (F2 and F3, FK506 5 mg) with the marketed SR