International Journal of Biological Macromolecules 83 (2016) 282–287

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Improved oral absorption of tacrolimus by a solid dispersion with hypromellose and sodium lauryl sulfate

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ARTICLE INFO

Article history:
Received 10 October 2015
Received in revised form 6 November 2015
Accepted 24 November 2015
Available online 28 November 2015

Keywords:
Tacrolimus
Solid dispersion
Hydroxypropyl methylcellulose
Sodium lauryl sulfate
Dissolution
Bioavailability

ABSTRACT

A novel surfactant-incorporated hydroxypropyl methylcellulose (HPMC) solid dispersion (SD) system was constructed in order to facilitate the release rate and oral absorption of tacrolimus (FK506), a poorly water-soluble immunosuppressant. Several emulsifiers including sodium lauryl sulfate (SLS), as drug release promotors, were employed with HPMC to fabricate SD using the solvent wetting method. The solid state characteristics using differential scanning calorimetry and X-ray powder diffraction, revealed that FK506 was molecularly distributed within all dispersions in amorphous form. The dissolution rates of FK506 in SLS-incorporated SDs were much higher than those in SDs prepared with HPMC alone, and even with stearoyl polyoxyl-32 glycerides or tocopheryl polyethylene glycol 1000 succinate. In particular, the greatest dissolution enhancement was obtained from the SD consisting of the drug, HPMC, and SLS in a weight ratio of 1:1:3, providing a 50-fold higher drug concentration within 15 min, compared with HPMC SD. *In vivo* absorption study in rats demonstrates that the optimized formula remarkably increased the oral absorption of FK506, providing about 4.0-fold greater bioavailability ($p < 0.05$) compared with the marketed product (Prograf®, Astellas Pharma). These data suggest that a novel SLS/HPMC SD may be an advantageous dosage form of FK506, boosting the dissolution and absorption in gastrointestinal tract.

1. Introduction

Tacrolimus (FK506), an immunosuppressant, has been clinically used in the prevention of organ rejection following hepatic and renal transplantation. It inhibits calcineurin by forming a complex with the FK506-binding protein, and was clinically demonstrated to be effective in the prevention of persistent refractory rejections in patients [1,2]. However, the oral therapy of FK506 has been relatively challenging due to its low and erratic intestinal absorption. Its poor water solubility (~5 μg/ml), pre-systemic metabolism by cytochrome P450, and P-glycoprotein-mediated efflux in the gastrointestinal tract, are predominantly attributed to its low and variable oral bioavailability (BA) [3,4]. To boost the dissolution rate and oral absorption of FK506, the originator formulated a solid dispersion (SD) system with hydroxypropyl methylcellulose (HPMC) (Prograf®, Astellas Pharma, US). The HPMC-based SD system increased the dissolution rate of the calcineurin inhibitor by reducing the drug particle size at the molecular level, thus changing the drug crystallinity to an amorphous state and hindering drug crystallization in aqueous medium [5–7]. However, the dissolution pattern and oral BA of the marketed product are still unsatisfactory, taking over 1 h for complete drug release and exhibiting a low BA (approximately 21%) with large intra- and inter-individual variability (4–89%) [8]. When considering the favorable absorbency of the compound in upper intestinal regions (*e.g.*, highest in the jejunum, intermediate in the ileum, and lowest in the colon) [3,4], a more rapid and profound drug release from the solid dosage form is desired in order to improve the oral absorption of FK506.

Over the last decade, amphiphilic materials including surfactants such as sodium lauryl sulfate (SLS), polysorbates, and d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS), have been introduced as carriers and/or additives in combination with polymeric materials for the preparation of SD systems to improve not only the physical stability of active compounds in the dispersions but also to boost the dissolution rate of poorly water-soluble compounds [9–11]. These surfactants with an amphiphilic character aid the physical miscibility of hydrophobic drugs with hydrophilic polymers, and reduce drug recrystallization in the dispersions. Moreover, these emulsifiers have been reported to improve drug wettability and prevent drug precipitation in the aqueous medium,

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http://dx.doi.org/10.1016/j.ijbiomac.2015.11.063
0141-8130/

by absorbing into the outer layer of drug particles and/or forming drug-loaded micelles [12]. SLS, a substance generally recognized as safe (GRAS), has been widely used as a solubilizer or co-carrier in solid systems to improve the solubility and release rate of Biopharmaceutical Classification System (BCS) II compounds, and the flow-ability of dispersion [13,14]. Previous study revealed that the incorporation of SLS into the sugar glass-based SD formula helped to preserve the high drug concentration in aqueous medium during the dissolution process, by preventing drug precipitation [15].

The aim of the present study was to formulate a novel surfactant-incorporated HPMC SD system to improve the dissolution rate and oral absorption of FK506. A supersaturable SD formula of FK506 was prepared with different kinds of surfactants including SLS using the solvent wetting technique. Each SD powder was evaluated for its physicochemical characteristics such as morphology, drug crystallinity, and *in vitro* dissolution profile under non-sink conditions. The pharmacokinetic profile of the optimized SD was comparatively evaluated with that of the marketed product (Prograf®) in rats, using a validated LC–MS/MS method.

2. Experimental

2.1. Materials

FK506 monohydrate was kindly provided by Chong Kun Dang Pharm (Seoul, Korea, purity over 99.0% w/w). Ascomycin (purity over 98% w/w), used as an internal standard (IS) for LC–MS/MS analysis, and SLS (purity over 98% w/w) were acquired from Sigma Chemical Co. (St. Louis, MO, USA). HPMC (Pharmacoat 606) was kindly provided from Shin-Etsu Chemical Co Ltd. (Tokyo, Japan). Poloxamer 188, Soluplus (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer), and Compritol 888 ATO (Glyceryl behenate) were kindly provided by BASF Co. (Ludwigshafen, Germany). Vit E TPGS was supplied by Abitec Co (Janesville, WI, USA). Gelucire 44/14 (Lauroyl polyoxyl-32 glycerides), 50/13 (Stearoyl polyoxyl-32 glycerides), Peceol (Glyceryl monooleate) and Transcutol P (diethylene glycol monoethyl ether) were kindly provided by Gattefosse (Saint Priest, France). Acetonitrile and methanol of HPLC grade were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade.

2.2. Aqueous solubility of FK506 in the presence of surfactants

The equilibrium solubility of FK506 in aqueous medium was determined by the shaking flask method, in the presence of different kinds of surfactants at a concentration of 1% (w/v). An excess of FK506 (5 mg) was added to a capped glass vial containing 3 ml distilled water with 10 mg/ml pharmaceutical excipients. Mixtures were vortexed for 5 min and then incubated for 24 h in a shaking incubator at 37 °C. Each vial was subsequently centrifuged at 13,000 rpm for 10 min. The supernatant was diluted with acetonitrile, and the drug concentration was analyzed by HPLC.

The quantitative FK506 analysis was performed using a Waters HPLC system (Model 515 pump, Model 717 plus auto sampler, Model 486 UV detector) equipped with a 4.6 mm × 150 mm ODS column (TSK-Gel ODS 80TM, Tosoh, Tokyo). The mobile phase consisted of distilled water, isopropyl alcohol, and tetrahydrofuran, at a volume ratio of 5:2:2. The flow rate was 1.0 ml/min, and the eluent was monitored at 220 nm. The peak of FK506 was identified at a retention time of 7.5 min.

2.3. Preparation of SD formulations by the solvent wetting technique

FK506 (100 mg) was dissolved in an appropriate quantity of ethanol. The amount of ethanol used was 2-fold higher than the total weight of the carrier (HPMC and each surfactant). The drug-containing ethanolic solutions were dropped onto different amounts of HPMC/surfactant mixture (100, 200, 300, and 400 mg) as described in Table 1. Solvents were then removed under a vacuum at 30 °C for 2 h. The prepared SD powders were pulverized for 10 min and then passed through a 200 μm microplate sieve.

Table 1
Compositions of the surfactant-incorporated HPMC SD formulations.

	F1	F2	F3	F4	F5	F6	F7	F8	F9
FK506 (mg)	5	5	5	5	5	5	5	5	5
HPMC (mg)	5	5	5	5	5	5	5	5	5
SLS (mg)	5	10	15	–	–	–	–	–	–
Gelucire 50/13 (mg)	–	–	–	5	10	15	–	–	–
Vit E TPGS (mg)	–	–	–	–	–	–	5	10	15

2.4. Morphological and physical characterization of SD formulations

2.4.1. Scanning electron microscopy (SEM)

The appearance of the drug powder, the physical mixture of the carrier materials, and the SD formula were observed using SEM (JSM-6510, Japan). The samples were placed on a copper grid using double-sided tape and coated with a thin layer of gold and palladium using an automatic magnetron sputter coater system (108Auto, Cressington Scientific Instruments Ltd., UK). The samples were then observed at an acceleration voltage of 10.0 kV.

2.4.2. X-ray powder diffraction (XRD)

Powder X-ray diffraction patterns of SD formulations were performed to verify the crystallinity state of FK506 in the formulations using an X-ray diffractometer (Ultima IV, Rigaku Corporation, USA) at room temperature. The diffraction pattern was measured over the most informative 2θ range, from 5° to 50°, using a step size of 0.02° and a scanning speed of 2 s/step.

2.4.3. Differential scanning calorimetry (DSC)

Thermal analyses were conducted using a DSC unit (DSC 50, Shimadzu Scientific Instruments, MD). Approximately 2 mg each sample was placed in aluminum pans and progressively heated at a scanning rate of 10 °C/min from 0 to 300 °C. Indium was used to calibrate the temperature scale and enthalpic response. A nitrogen flow rate of 20 ml/min was used for each differential scanning calorimetry run.

2.5. Dissolution studies

The dissolution profiles of each formulation under non-sink conditions were evaluated according to the USP apparatus II (paddle) method equipped with a dissolution testing station and a heater (Hanson, USA). Each formulation, containing 50 mg FK506, was located in the glass vessel containing 500 ml dissolution medium (simulated gastric juice, intestinal fluid, and distilled water) maintained at 37.0 ± 0.5 °C and stirred at 50 rpm. Approximately 5 ml dissolution medium was withdrawn and replaced with fresh and pre-warmed dissolution medium. Withdrawn aliquots were then centrifuged at 13,000 rpm for 10 min and the supernatants were diluted with mobile phase for HPLC analysis.

2.6. In vivo *oral absorption study*

2.6.1. Animals and drug administration

Sprague-Dawley rats (male; 200–230 g; 6–7 weeks) purchased from Orient Bio (Kyungki-do, Korea) were housed in