International Journal of Pharmaceutics 513 (2016) 255–261

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Development of solidified self-microemulsifying delivery systems with enhanced stability of sirolimus and extended release

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ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 1 September 2016
Accepted 10 September 2016
Available online 11 September 2016

Keywords:
Sirolimus
Self-microemulsifying drug delivery system
Tablet
Stability
Extended release

ABSTRACT

The application of sirolimus (SRL) as immunosuppressive agent is hampered by its poor water solubility and narrow therapeutic range. The self-microemulsifying drug delivery system (SMEDDS) succeeded in improving the solubility of SRL in our previous work. In this study, the formulation of the SMEDDS was further optimized by investigating the influence of the excipients including the media, antioxidant and organic acid. It was demonstrated that addition of 0.20% of citric acid in SMEDDS most efficiently promoted the stability of SRL under high temperature ($40 \pm 2\,^{\circ}C$), high humidity (relative humidity $90 \pm 5\%$) or strong light irradiation (4500 ± 500 lx). SMEDDS absorbed by microcrystalline cellulose (MCC) was mixed with hydroxypropyl methylcellulose (HPMC) to prepare tablets. The optimal formulation composed of 15% of HPMC 100 LV with hardness of 120 N, which had a sustained release of 12 h. Results of X-ray powder diffraction and differential scanning calorimetry demonstrated that SRL in the tablets was in amorphous or molecularly dispersed state. The SMEDDS-tablets presented as promising substrates for water insoluble drugs with enhanced stability and extended release.

1. Introduction

Sirolimus (SRL), also known as rapamycin, is one of the most widely used immunosuppressive agents for organ transplantation (Zhao et al., 2016). But the application of SRL is hampered by its poor solubility and narrow therapeutic range. Firstly, SRL belongs to biopharmaceutics classification system (BCS) class II drug category, indicating its low solubility and high permeability. So the relatively low bioavailability is mainly caused by its poor solubility (Khan et al., 2015; Vasquez, 2000). The product information revealed that the mean bioavailability was estimated to be approximately 14% and 17% for the commercial oral solution and tablets, respectively (Rapamune product literature, 2009).

Secondly, because of the narrow therapeutic range of SRL, side effects including hyperlipemia and cytopenias are quite common (Ventura-Aguiar et al., 2016). Especially, most of the serious side effects are dose-dependent. Adverse events could be found in most of the patients treated with high level of SRL (Thibodeau et al., 2013). Thus a sustained release, which is not offered by the commercial tablets, is desired to keep the blood concentration of SRL in the therapeutic range. In conclusion, a favorable delivery system with improved solubility and a sustained release behavior of SRL is needed to promote the bioavailability and decrease side effects.

Various studies have been focused on improving the solubility of SRL. Nano-medicine presents promising technical in the future. Compared with nanoparticles, lipid based emulsions are more favorable and are commercially available for oral administration. The self-microemulsifying drug delivery system (SMEDDS) is one of the most promising emulsions (Dokania and Joshi, 2015). The SMEDDS is usually composed of oil phase, emulsifier and co-emulsifier (Rahman et al., 2013). After oral administration, the SMEDDS can form microemulsions of nano-sized droplets (<300 nm) upon body fluids in the gastrointestinal tract. The SMEDDS are excellent delivery systems for hydrophobic drugs including atorvastatin calcium, finasteride, acyclovir et al. (Fagir et al., 2015; Kazi et al., 2015; Yeom et al., 2016). The commercial ciclosporin (Sandimmun Neoral®), ritonavir (Norvir®) and saquinavir (Fortovase®) are based on SMEDDS. Moreover, the SMEDDS could be solidified to prepare tablets and dropping pills

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http://dx.doi.org/10.1016/j.ijpharm.2016.09.035
0378-5173/

for better production, transportation, storage and administration (Cerpnjak et al., 2015; Guan et al., 2016).

SMEDDS present promising substrates for SRL. According to our previous study, SRL-loaded SMEDDS were successfully developed and were further solidified to prepare pellets. The oral bioavailability was improved as compared with the commercial SRL tablets (Hu et al., 2012). But the SRL was completely released from the pellets in 2 h, causing a high blood concentration. To protect the patients from rejection and toxicity in advance, a sustained release system will be favorable to achieve a steady blood concentration.

This study was focused on optimization of the SMEDDS for improved stability of SRL, and development of SMEDDS-based tablets for extended in vitro release.

2. Materials and methods

2.1. Materials

SRL was purchased from Kerui Pharmaceutical Co., Ltd. (Fuzhou, China). Transcutol P, Labrafil M 1944CS and Labrasol were gifted by Gattefossé (Brittany, France). Solutol HS 15 was obtained from BASF (Ludwigshafen, Germany). Cremophor EL was purchased from Fengli Jingqiu Pharmaceutical Co., Ltd. (Beijing, China). Tween 80 was purchased from Croda (Cowick Hall, England). L-Ascorbic-acid (L-A) and *tert*-butyl hydroquinone (TBHQ) were purchased from Jonln Industrial Co., Ltd. (Shanghai, China). Citric acid was purchased from Kefeng Chemical Reagent Co., Ltd. (Shanghai, China). Hydroxypropyl methylcellulose (HPMC) was purchased from Colorcon Coating Technology Co., Ltd. (Shanghai, China). All other reagents were of analytic grade.

2.2. Preparation and characterization of SMEDDS

2.2.1. Preparation of SMEDDS

The preparation of SMEDDS was based on our previous study (Hu et al., 2012). The optimized formulation consisted of Labrafil M 1944CS (oil phase), Cremophor EL (emulsifier) and Transcutol P (co-emulsifier). SRL had much higher solubility in Transcutol P than that in Labrafil M 1944CS and Cremophor EL. So SRL (0.1 g) was first dissolved in Transcutol P (1.92 g) by sonication, after which Labrafil M 1944CS (2.24 g) and Cremophor EL (3.84 g) were added under stirring. A transparent solution of SMEDDS was obtained then.

2.2.2. Stress test

Stability of SRL is the fundamental factor for the application of the SRL-SMEDDS. To evaluate its stability, stress tests were performed based on the Chinese Pharmacopoeia (The State Pharmacopoeia Commission of P. R. China, 2015). Raw SRL powders and SRL-SMEDDS were placed in conditions of high temperature ($40 \pm 2\,^{\circ}C$), high humidity (relative humidity $90 \pm 5\%$) or strong light irradiation (4500 ± 500 lx). At day 0, 5 and 10, the SRL remaining in the samples were analyzed by HPLC.

2.2.3. Influence of antioxidant

The influence of antioxidant towards the stability of SRL in SMEDDS was investigated. L-A, vitamin E and TBHQ are the most usually used antioxidants. SMEDDS with vitamin E (1%), L-A (1%), TBHQ (1%) or L-A (0.5%) & TBHQ (0.5%) were prepared and tested at 40 °C, respectively. The contents of SRL at day 0, 5 and 10 were analyzed by HPLC.

2.2.4. Influence of pH

To investigate the influence of pH, SRL dissolved in methanol was diluted with PBS of pH 2.0, 4.0, 4.5, 5.0, 5.8, 6.8, 7.4 and 7.8. The samples were kept at room temperature. At 0, 2, 4, 6, 8, 10, 12 and 24 h samples were collected and analyzed by HPLC. Citric acid was used to adjust the pH of the SMEDDS. 0.125%, 0.20% and 0.25% of citric acids were added into the SMEDDS, which were incubated at 40 °C for 10 days and analyzed by HPLC.

2.2.5. Characterization of the optimal SMEDDS

To study the formation of microemulsion, 0.5 mL of SMEDDS were added into 50 mL of deionized water. The mixture was incubated at 37 °C under stirring (50 rpm). The time of complete formation of the microemulsion was determined as the self-emulsifying time (t). The size and polydispersity index (PDI) of the microemulsion were tested by a particle sizing system (Nicomp380, PSS Inc., USA).

2.3. Preparation and characterization of the tablets

2.3.1. Preparation of tablets

MCC (40.8 g) was used to absorb the SMEDDS at a weight ratio of 2:1 (MCC:SMEDDS). Then HPMC was blended with the mixture, and a single punch tablet machine (YPD-200C, Huanghai Co., Ltd., China) was used to produce the tablets (560 mg per tablet).

2.3.2. Stability of tablets

Stress test was performed to evaluate the stability of SRL in the tablets. Tablets were incubated in conditions of high temperature ($40 \pm 2\,^{\circ}C$), high humidity (relative humidity $90 \pm 5\%$) or strong light irradiation (4500 ± 500 lx) using a multi-Drug Stability Test Chamber (SHH-SDT, Ysei Experimental Instrument Factory, China). Afterwards, the tablets were further kept in blister packs and were evaluated by stress test again.

2.3.3. Optimization of tablets

The tablets were optimized to obtain a sustained release of SRL. A dissolution tester (RCZ-6BZ, Huanghai Co., Ltd., China) was used. The types (K100LV, K4M, K15M and K100M) and contents (10%, 15% and 20%, w/w) of HPMC, and the hardness (100 N, 120 N and 140 N) of the tablets were regulated for the dissolution test. Six tablets were added into 250 mL of water (0.4% SDS) at 37 °C. At each time point (2, 4, 6, 8, 10 and 12 h), 5 mL of media was collected and determined by HPLC. Equal volume of fresh media was added at the same time. An optimal formulation was achieved based on the in vitro release behavior of SRL.

Three batches of the optimized tablets were prepared and the release profiles were compared using the similarity factor ($f2$). The $f2$ was calculated according to the equation $f2 = 50 \times \lg\{[1 + (1/n)\Sigma(R_t - T_t)^2]^{1/2} \times 100\}$. Rt and Tt referred to the average cumulative release at a certain time point, and n meant the number of time points. The release profiles could be considered as being similar when $50 < f2 < 100$. To investigate the release kinetics, data were plotted in various kinetic models including zero order, first order, Higuchi and Ritger-Peppas models.

2.3.4. Characterization of the tablets

2.3.4.1. Differential scanning calorimetry (DSC). Thermal investigation was performed by DSC (STA449C, Netzsch, Germany) to test the physical state of SRL in SMEDDS and tablets. Samples of raw SRL, blank SMEDDS-tablet, physical mixture of SRL and blank SMEDDS-tablet, and SRL-SMEDDS-tablets were tested. The samples were placed in aluminum pans and were heated from 25 to 300 °C with a heating rate of 10 °C/min under a nitrogen atmosphere.

2.3.4.2. X-ray powder diffraction (XRPD). The samples were further tested by XRPD. Raw SRL, blank SMEDDS-tablet, physical mixture of SRL and blank SMEDDS-tablet, and SRL-SMEDDS-tablets were