Solid dispersion-based pellet for colon delivery of tacrolimus through time- and pH-dependent layer coating: preparation, *in vitro* and *in vivo* studies

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The intent of the present investigation is to develop and evaluate colon-specific coated tacrolimus solid dispersion pellet (SDP) that retards drug release in the stomach and small intestine but progressively releases in the colon. Tacrolimus-SDP was prepared by extrusion-spheronization technology and optimized by the micromeritic properties including flowability, friability, yields and dissolution rate. Subsequently, the pH-dependent layer (Eudragit L30D55) and time-dependent layer (Eudragit NE30D and L30D55) were coated on the SDP to form tacrolimus colon-specific pellets (CSP) using a fluidized bed coater. Under in vitro gradient pH environment, tacrolimus only released from CSP after changing pH to 6.8 and then quickly released in the phosphate buffer solution of pH 7.2. The Cmax of CSP was 195.68 ± 3.14 ng/mL at Tmax 4.5 ± 0.24 h where as in case of SDP, the Cmax was 646.16 ± 8.15 ng/mL at Tmax 0.5 ± 0.03 h, indicating the ability of CSP targeted to colon. The highest area under the curve was achieved 2479.58 ± 183.33 ng·h/mL for SDP, which was 2.27-fold higher than tacrolimus suspension. However, the best biodistribution performance was achieved from CSP. In conclusion, SDP combining of pH- and time-dependent approaches was suitable for targeted delivery of tacrolimus to colon.

Keywords: Tacrolimus. Solid dispersion. Pellet. Oral colon-specific. Biodistribution.

INTRODUCTION

Ulcerative colitis (UC) is a type of inflammatory bowel disease related to the autoimmune system. The outbreak of acute UC has high mortality. The chronic UC generally deteriorates to colon cancer and eventually requires surgery (De Toni *et al*., 2015). Currently, the medicines of UC treatment include non-steroidal anti-inflammatory drugs, glucocorticoids, synthetic immunosuppressants and biologic agents (Park, Jeen, 2015). Tacrolimus is a type of macrolide lactone with potent immunosuppressive activity, which is used for organ rejection prophylaxis in liver, kidney and small intestine transplantation (Fang, Ma, Gui, 2015). It potentially inhibits calcineurin phosphatase activity and prevents the generation of nuclear factor of activated

T cells (NF-AT). NF-AT activates the cytokine genes related to interleukin (IL)-2, tumor necrosis factor-α and interferon-γ in T lymphocytes. The immunosuppressive effects of tacrolimus are partially mediated by inhibition of IL-2 secretion and decrease the number of IL-2 receptors for T lymphocyte activation (Navas-Lopez *et al*., 2014). The steroid resistance is related to the high levels of IL-2 release and intrinsic properties of IL-2 receptor in steroid resistant lymphocytes (Ogata *et al*., 2006). Therefore, tacrolimus will induce steroid-refractory UC remission, and is considered as a second-line medicine in severe inflammatory bowel disease treatment (Miyoshi *et al*., 2013). In a study conducted by Landy *et al*. (2013) a total of 25 patients with refractory UC had achieved and maintained clinical remission by oral tacrolimus in six months (Landy *et al*., 2013).

Tacrolimus is a lipophilic molecule consisting of 23-member macrolide lactone. Therefore, it is limited in oral absorption in gastrointestinal tract, which induces the variable and poor oral bioavailability (Tajdaran *et al*.,

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2015). In order to improve the bioavailability of tacrolimus, we had developed the tacrolimus-solid dispersion (SD) through solvent evaporation method in previous studies (Fang *et al*., 2016). SD generally provides a high maximum blood concentration (*C*max) in blood at early time period following oral administration, and the duration time of effective blood concentrations is relatively short (Tsunashima *et al*., 2016). On the other hand, tacrolimus in non-specific delivery systems is initially absorbed into blood circulation, and then is redistributed in colon tissue. This causes the effective drug concentration low and variable in colon tissue.

Oral colon-specific delivery systems (OCDDS) do not release drugs in stomach and small intestine after oral administration, but specifically release drug in cecum or colon part (Bansal *et al*., 2014). The commonly applied dosage forms of OCDDS are tablets, capsules, gels and pellets (Vemula, 2015a; Vemula, 2015b; Dodoo *et al*., 2017; Bose, Elyagoby, Wong, 2014). The mechanisms of OCDDS mainly involve the following (Bansal*et al*., 2014). (1) pH-dependent OCDDS, carrier material was disintegrated in the colon based on the specific gradient pH in the gastrointestinal tract (stomach pH: 0.9-1.5, small intestine pH: 6.0-6.8, colon pH: 6.5-7.8. (2) Time-dependent OCDDS, carrier material was disintegrated in the colon based on constant transit time of carrier in the stomach and small intestine, generally arrived to the colon part after 4-5 h upon oral administration. (3) Microbial-dependent OCDDS, carrier materials were degraded by the specific enzymes secreted by colonic bacteria in the colon part. Among these approaches, microbial-dependent OCDDS were limited on the choice of specific carrier materials (Xu *et al*., 2014). The pH-dependent OCDDS approach is simple for operation, but there some researches prove that infeasible by using only one strategy as OCDDS in different physiological and pathological conditions for colon delivery. Therefore, the pH-dependent OCDDS was assessed in combination with time-dependent OCDDS to ensure drug release site accurately under different physiological conditions (Vemula, Veerareddy, Devadasu, 2015; Veerareddy, Vemula, 2012). For example, Park *et al*., reported that an enteric coated multiple-unit tablet system of bisacodyl consisting of Eudragit S/L-based pH-dependent and time-dependent controlled release polymer of Eudragit RS provided selective drug release in colon. The pharmacokinetic evaluation in rabbits suggested that drug absorption from the time- and pH-dependent tablet was effectively retarded in stomach and small intestinal with lowered systemic exposure compared to marked product of Dulcolax®, but profound drug liberation was achieved in colon part (Park *et al*., 2017).

Considering the disadvantages of tablet manufacturing, including capping, laminations, variations in weight and the processing cost, an attempt was made to develop tacrolimus SD-based controlled-release pellets. Pellet is a kind of multiple-dose drug delivery system, which has the advantages of reducing gastrointestinal irritation, non-affecting dietary rhythms, nice reproducibility of pharmacokinetics, *etc*. Furthermore, pellets are spherical small-unit delivery systems with the diameter of 0.5-1.5 mm. The research indicated that small-unit delivery systems like pellets have a longer retention time compared to big-unit delivery systems in gastrointestinal tract (Dhandapani, 2005). To our knowledge, no reports have investigated the combination of OCDDS and SD formulations for tacrolimus. Therefore, the aim of this study was to design a novel double enteric coated pellet of tacrolimus for colon targeted delivery. We initially developed tacrolimus solid dispersion pellet (SDP) and then optimized the formulation consisting of Eudragit NE30D and Eudragit L30D55 as time-dependent layer, and Eudragit L30D55 as pH-dependent layer for the tacrolimus colon-specific pellet (CSP) to achieve OCDDS. Eudragit is a water-soluble coating material suitable for industrial production. At present, colon-targeted drugs with Eudragit coating are available on the market. Marketed mesalazine-loaded pellet formulation of Salofalk® granule was targeted to colon site by pH- and time-dependent coating layer of Eudragit L100 and Eudragit NE (Schellekens *et al*., 2007). Furthermore, the oral bioavailability of tacrolimus incorporated into SDP and CSP were evaluated. The pharmacokinetics and biodistribution profiles of tacrolimus-SDP and -CSP were comparatively evaluated in rats using tacrolimus suspension as a reference.

MATERIAL AND METHODS

Material

Tacrolimus was obtained from Meryer Chemical Technology Ltd (Shanghai, China). Eudragit NE30D and Eudragit L30D55 dispersions were donated by Evonik Corporation (Germany). Starch was obtained from Dongyuan Pharm Ltd (Liaoning, China). Microcrystalline cellulose (MCC), carboxymethyl cellulose sodium (CMC-Na), poly vinyl pyrrolidone (PVP) K30, carboxymethyl starch sodium (CMS-Na), low-substituted hydroxypropyl cellulose (L-HPC), silica gel and talc were obtained from Anhui Sunhere Pharmaceutical Excipients Ltd (Huainan, China). Triethyl citrate was obtained from Aladdin Corporation (Shanghai, China). Methanol and

acetonitrile (HPLC gradient grade) were obtained from Merck (Shanghai, China), Ascomycin was donated by Shanghai Xuhui District Central Hospital. Purified water from a Milli-Q system (Millipore,Bedford, MA, USA) was used throughout the experiment. All other materials used were of standard pharmacopoeia grade or analytical reagent grade.

Preparation of tacrolimus solid dispersion

We had successfully prepared tacrolimus-SD by the solvent evaporation method in previous research (Fang *et al*., 2016). The results of fourier transform infrared spectroscopy, differential scanning calorimetry, scanning electron microscopic pictures of SD powder indicated that tacrolimus existed in an amorphous state with hydroxypropyl methyl cellulose (HPMC) in SD, resulting in *in vitro* dissolution enhancement compared to the marketed tacrolimus powder (Fang *et al*., 2016). In brief, tacrolimus and HPMC were accurately weighed and tacrolimus was completely dissolved in the mixture solution containing ethanol and dichloromethane (1:1, v/v). Then, HPMC was swollen and added by the mixture solution. The mixed solvent was evaporated under a water bath at 50 °C and dried in a vacuum drying oven at 45 °C for 24 h. After drying, SD powders were pulverized and classified by a sieve of 180 μm.

Preparation and optimization of tacrolimus solid dispersion pellets

Tacrolimus-SDP was prepared by an extrusion-spheronization process. Tacrolimus-SD and all the excipients were firstly sieved through a 0.154 mm mesh. The powders were blended and wetted with 5% PVP K30 by 30% ethanol until a homogeneous mass was obtained. Subsequently, the wet mass was extruded in an extruder (Enger E-50/ R-250, Chongqing Enger Graulating and Coating Technology Co., Ltd, China) using a 0.8 mm mesh. The extrudates were subsequently spheronized at 500 rpm for 5 min (Enger E-50/R-250, Chongqing Enger Graulating and Coating Technology Co., Ltd, China) and dried in a vacuum oven at 50 °C for 24 h.

Micromeritic properties of tacrolimus solid dispersion pellets

Micromeritic properties of pellets were determined prior to the coating process for SDP formulation optimization. Flowability, friability and yield were evaluated in an uncoated SDP sample.

Flowability was evaluated by angle of repose (φ). The pellets (5 g) were placed on a smooth and flat plate. One side of plate was lifted slowly. The angle of repose was formed by inclined surface and horizontal line when 90% of pellets start rolling.

Friability (F) was determined to ensure that the pellets could be coated without loss of weight. The less friability of pellets was contributed to further coating. This was measured by comparing the pellets weight before and after a fluidized test at 0.8 m^3/min air flow rate for 10 min in the fluidized bed device. The pellets (20 g) were accurately weighed before the test.

$$F = \frac{\text{weight before test-weight after test (g)}}{\text{weight after test (g)}}$$

The obtained pellets were sieved by 700-880 μm screens. The yield (Y%) was obtained using the following equation.

$$Y\% = \frac{\text{weight of pellets (g)}}{\text{weight of drug and excipients before preparation (g)}} \times 100\%$$

Flowability, friability and yield tests were implemented in triplicate for each pellet batch and the results were averaged.

Time- and pH-dependent layer coating of tacrolimus colon-specific pellets

The time-dependent layer coating solution was prepared by mixing talc (anti-coherent agent) and the water dispersions of Eudragit NE30D and Eudragit L30D55. The pH-dependent layer coating solution was prepared by mixing talc (anti-coherent agent), triethyl citrate (plasticizer) and the dispersion of Eudragit L30D55. The mixed suspension was stirred until a uniform suspension was obtained (about 80 rpm, 30 min). The final solid content was 15% in the suspension. The coating solutions were sieved by 0.45 mm mesh before use.

Tacrolimus-loaded pellets were coated with time- and pH-dependent layer using a fluidized bed coater with a bottom sprayer (MinLab XP, DIOSNA Co., Ltd, Germany). The pellets were fluidized and exposed on a hot air. The resulted coating solutions were layered onto the surface of pellets. The process parameters of fluidized bed coater were as follows: air flow speed of 1.3 m^3/min; atomization pressure of 0.8 MPa; peristaltic pump speed of 10 Hz; nozzle diameter of 0.5 mm, inlet temperature of 26 °C (time-dependent layer) / 40 °C (pH-dependent layer). The coating solution was stirred constantly to

maintain a uniform state and prevent talc deposition in coating process. After coating, the coated-pellets were further fluidized for 3 min and subsequently dried in an oven for 12 h at 40 °C.

In vitro release kinetic studies

All *in vitro* release tests were conducted with Chinese Pharmacopoeia (ChP) (Edition 2015) apparatus of intelligent dissolution instrument (RC1208D, TDTF Co., Ltd, Tianjin, China) at 37 °C. ChP paddles rotating at a speed of 75 rpm were used. Coated-pellets (5 mg tacrolimus) were soaked in 750 mL of HCl solution (0.1 mol/L) at pH 1.2 for 2 h, then switched to 900 mL of ChP phosphate buffer solution (PBS) at pH 6.8 and pH 7.2 for the remaining 3 h and 7 h, respectively. At given time intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h, 4 mL samples were withdrawn and filtered through a 0.45 μm solvent resistant filter. The equal amount of 4 mL release medium was complemented. The *in vitro* release experiments were performed in triplicate.

The concentrations of tacrolimus in each filtered sample were determined by the HPLC system (Agilent 1200 series HPLC system, G1312A Binary Pump, G1314B Variable Wavelength Detector, Agilent Co., Ltd, USA). The determination of tacrolimus was performed using a Kromasil100-5C18 column (250 mm × 4.6 mm, 5 μm, Akzo Nobel Co., Ltd, Sweden) at a wavelength of 220 nm at 40 °C. The mobile phase consisted of acetonitrile-distilled water (70:30, v/v) operating at a flow rate of 1.0 mL/min. The injection volume of sample was 20 μL. All experiments were performed in triplicate.

The data obtained from the *in vitro* release studies were performed using DDsolver software following below equation (Zhang *et al.*, 2010). The data of *in vitro* release were fitted to Zero order, First order, Weibull and Higuchi models to explore the pattern and the release mechanisms from the formulations. Koresmeyer–Peppas model was used to understand the mechanism of drug release from the formulations. The mean dissolution time (*MDT*) was calculated by the following equation (Veerareddy, Vemula, 2012):

$$MDT = \frac{\sum_{i=1}^{i=n} tmid \times \Delta M}{\sum_{i=1}^{i=n} \times \Delta M}$$

where *i* is the dissolution sample number, *n* is the number of dissolution sample time, t_{mid} is the time at the midpoint between *i* and *i = 1*, and *ΔM* is the amount of drug dissolved between *i* and *i = 1*.

T10% and T80% (time in hours to take 10% and 80% drug release, respectively) were calculated to clarify the colon-specific release from CSP (Vemula, Veerareddy, 2013). In the pair-wise approach, the similarity factor (f_2) using the mean percentage cumulative released values was performed by using the following equation (Vemula, 2015a):

$$f_2 = 50 \lg \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} \left(R_t - T_t \right)^2 \right]^{-0.5} \times 100 \right\}$$

where *n* is the number of time points, R_t and T_t are cumulative release of reference and test preparation at time point *t,* respectively. The value of f_2 ranges between 0 and 100. When the test and reference profiles are identical, the value of f_2 approaches to 100, and as the value decreases, the dissimilarity between the profiles increases.

In vivo pharmacokinetic and biodistribution studies of tacrolimus colon-specific pellets

Animal experiments

Male Sprague-Dawley rats were provided by Experimental Animal Center of Anhui Medical University (Hefei, China). Animals were kept under 12 h light / dark cycles with free access to food and water, and were acclimatized for at least 5 days prior to the experiment. All animal protocols were approved by the Animal Ethic committee of Anhui University of Chinese Medicine (Approval No. KJ: 013-16, KJ: 013-18), and experiments were conducted in accordance with the NIH "Principles of laboratory animal care" guidelines.

For *in vivo* pharmacokinetic studies, eighteen healthy Sprague-Dawley rats (250 ± 20 g) were randomly divided into three groups (n = 6) and fasted 12 h prior to the gavage administration of drug formulations. Marketed tacrolimus powder, tacrolimus-SDP, and optimized tacrolimus-CSP were dispersed in normal saline of 10 mg/mL. The drugs were administered to rats by gavage through a syringe fitted with a flexible oral-zoned catheter at a tacrolimus dose of 3 mg/kg. For tacrolimus suspension treatment group, blood samples were withdrawn from the retro-orbital plexus using heparin sodium-treated syringes at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 12 and 24 h after drug dosing. For tacrolimus-SDP and tacrolimus-CSP treatment groups, blood samples were withdrawn from the retro-orbital plexus using heparin sodium-treated syringes at 1, 1.5, 2, 3, 4, 4.5, 5, 6, 8, 10, 12 and 24 h after drug dosing. The blood samples were kept below -20 °C until analysis by a UPLC assay as described below.

For the tacrolimus biodistribution studies, seventy-two healthy Sprague-Dawley rats (250 ± 20 g) were

randomly divided into three groups (n = 24): tacrolimus suspension group, tacrolimus-SDP group, and tacrolimus-CSP group. Rats were deprived foods in 12 h prior to dosing. The drugs were gavaged to rats through a syringe fitted with a flexible oral catheter at a tacrolimus dose of 3 mg/kg. For each group, rats (n = 3) were anesthesia by 5% chloral hydrate and sacrificed at pre-determined time points of 2, 4, 6, 8, 10, 12, 16 and 24 h following drug dosing, respectively. The rat tissues (stomach, proximal small intestine, distal small intestine, cecum and colon) were removed quickly and cautiously. Next, the samples were cut into tiny pieces, weighed and homogenized with the normal saline using a homogenizer (T18 ULTRA-TURRAX, IKA Co., Ltd, Germany). After centrifuging at 3500 rpm for 15 min at 4 °C, 200 μL of supernatants samples were collected and stored at -20 °C until UPLC analysis.

Determination of tacrolimus in rat blood and tissue samples

Tacrolimus concentrations in blood and tissue samples were determined using a UPLC method. For sample preparation, blood or tissue supernatants samples (200 μL), NaOH solution (0.2 mol/L, 200 μL) and internal standard solution (ascomycin, 200.7 ng/mL, 100 μL) were added. The mixed samples were then extracted with methyl tert-butyl ether (3 mL), by shaking on a mechanical shaker (TYXH-II, Shanghai Green Instrument Co., Ltd, China) for 3 min. After centrifugation at 3000 rpm for 3 min at 4 °C, the upper organic layer was removed and evaporated to dryness under nitrogen gas at 50 °C. The residue was dissolved in 100 μL of the mobile phase. After centrifugation for 12000 rpm for 10 min at 4 °C, 2 μL of sample supernatant was analyzed by UPLC system (ACQUITY UPLC H-Class system, quaternary pump, Tunable UV detector, Waters Co., Ltd, USA). Chromatography was performed using an ACQUITY UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm, Waters Co., Ltd, USA) at 45 °C, while data analysis was performed using the Empower 3 chromatography data software (Waters Co., Ltd, USA). The detection wavelength of tacrolimus was at 220 nm. The mobile phase was a mixture of acetonitrile and 0.1% phosphoric acid solution (75:25, v/v) and its flow rate was at 0.3 mL/min.

Pharmacokinetic analysis

Cmax and its time (Tmax) were directly obtained from the mean blood concentration-time curve. Some other important pharmacokinetic parameters, *e.g.* biological half-life ($t_{1/2}$), mean residence time (MRT) and area under the curve (AUC), were calculated using DAS 2.0 pharmacokinetics software.

In vitro-in vivo correlation

To generate *in vitro-in vivo* correlation (IVIVC), the *in vitro* cumulative percent of tacrolimus release of optimized CSP formulation was compared against the extent of *in vivo* absorption, *i.e.*, cumulative AUC values of the same formulation (Vemula, 2015c).

Statistics analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed by SPSS 17.0 statistical software using one-way ANOVA test. The differences are assumed to be statistically significant at $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Preparation and optimization of tacrolimus solid dispersion pellets

As a biopharmaceutics classification system (BCS) class II drug, the clinical efficacy of tacrolimus was limited due to its poor water solubility (5-8 μg/mL), which is responsible for its low oral bioavailability (Wang, Gan, Zhang, 2011). SD technology has been widely used to increase the water solubility of lipophilic drugs. Tacrolimus-SD had been successfully developed using the solvent evaporation method in previous research (Fang *et al*., 2016). The matrix of tacrolimus-SD was HPMC and the weight ratio of HPMC: tacrolimus was 5:1. The tacrolimus and soluble matrix of HPMC were formed into a complex so that tacrolimus lost its original crystal structure and presented as an amorphous state in the SD. *In vitro* experiments showed that the dissolution of tacrolimus-SD was significantly faster than that of single tacrolimus or physical mixtures of tacrolimus/HPMC (Fang *et al*., 2016). For BCS class II drugs, the maintenance of supersaturation sate facilitates drug release from the formulations. Yamashita *et al*., reported that the C=O functional groups and O-H of tacrolimus could interact with the functional groups of HPMC, which contributed to the maintenance of supersaturation state of tacrolimus when it was released from the SD (Yamashita *et al*., 2003).

Extrusion-spheronization is a kind of economical and practical technology with high yields, good repeatability and suitable for industrial production. The preparation

of wet mass takes a vital influence on pellet formulation. The excipients with appropriate plasticity and water retention are necessary for wet mass preparation. During the extrusion-spheronization process, the impacts of drug-loading rate and the polymers of excipients, including filler, disintegrate agent, on SDP micromeritic properties were investigated. Different polymers in each excipient category were examined as follows: filler (lactose, starch, chitosan, MCC), disintegrate agent (PVP, CMC-Na, CMS-Na, L-HPC) and four levels of drug-loading rate (5%, 10%, 15%, 20%), to evaluate the quality of tacrolimus-SDP. The formulations of tacrolimus-SDP with different compositions were given in the Table I and their micromeritic property characterization of angle of repose, friability, yield and dissolution rate is shown in the Table II.

The characterization results of tacrolimus-SDP prepared by various filler agents (F1, F2, F3, F4, F5) suggested that spherical shape pellets with less rod and dumbbell shape were prepared by the formulations of F3, F4 and F5. Using lactose alone in F4 caused the conglutination of pellets in spheronization process. The yields of F3 and F5 both were above 70%, and had appropriate degrees of angle of repose, friability. Considering the hydrophobicity of MCC, the combination of MCC and lactose was applied to improve pellet dissolution rather than using MCC alone as the filler agent. The weight ratios of lactose and MCC were further investigated in 1:1.5, 1:1 and 1.5:1 (F14, F15, F16). The appearance of pellets was spherical shape in F14 formulation (lactose: MCC=1:1.5); the yield and dissolution rate were 76.8% and 83.4%, respectively. The results indicated the yield decreased as the proportion of lactose increased in the SDP.

The micromeritic characterization and the dissolution studies were applied by tacrolimus-SDP with various disintegrate agents (F6, F7, F8, F9). The results showed that the F8 with CMS-Na had the highest dissolution rate. When water infiltrated into the interior of pellet and absorbed by CMS-Na, CMS-Na had powerful swelling ability and eventually caused pellet collapse, which accelerated disintegration process of SDP. CMS-Na is an appropriate disintegrate agent for tacrolimus-SDP. The proportion of 4% CMS-Na was adopted in the formulation. Considering the absorption difference based on the individual physiological or pathological gastrointestinal tract conditions, if the SDP core of pellet was not immediately disintegrated and released drug in targeted site *in vivo*, the drug may excrete from the body without sufficient absorption. Therefore, the SDP

TABLE I - Composition of tacrolimus-SDP

	Tacrolimus (g)	Lactose (g)	Starch (g)	Chitosan (g)	MCC (g)	CMC-Na (g)	CMS-Na (g)	L-HPC (g)	PVP K30 (g)
F1	0.8	2.8	3.84	-	-	0.32	-	-	-
F2	0.8	2.8	-	3.84	-	0.32	-	-	-
F3	0.8	2.8	-	-	3.84	0.32	-	-	-
F4	0.8	6.64	-	-	-	0.32	-	-	-
F5	0.8	-	-	-	-	0.32	-	-	-
F6	0.8	2.8	-	-	3.84	-	-	-	0.32
F7	0.8	2.8	-	-	3.84	0.32	-	-	-
F8	0.8	2.8	-	-	3.84	-	0.32	-	-
F9	0.8	2.8	-	-	3.84	-	-	0.32	-
F10	0.4	3.2	-	-	3.84	-	0.32	-	-
F11	0.8	2.8	-	-	3.84	-	0.32	-	-
F12	1.2	2.4	-	-	3.84	-	0.32	-	-
F13	1.6	2.0	-	-	3.84	-	0.32	-	-
F14	0.8	2.66	3.98	-	-	-	0.32	-	-
F15	0.8	3.32	3.32	-	-	-	0.32	-	-
F16	0.8	3.98	2.66	-	-	-	0.32	-	-

Note: the symbol of "-" expresses data not available. CMC-Na: carboxymethyl cellulose sodium; CMS-Na: carboxymethyl starch sodium; L-HPC: low-substituted hydroxypropyl cellulose; MCC: microcrystalline cellulose; PVP: poly vinyl pyrrolidone.

TABLE II - Micromeritic properties and dissolution rate of tacrolimus-SDP

	Morphology	Angle of repose (φ)	Friability (F)	Yield (Y%)	Dissolution rate (%)
F1	Rod and dumbbell shape	19.2	1.38	48.8	-
F2	Rod and dumbbell shape	21.7	1.46	41.9	-
F3	Spherical pellet, less rod and dumbbell shape	15.8	0.72	71.1	-
F4	Spherical pellet but conglutination in spheronization process	18.5	0.96	67.5	-
F5	Spherical pellet, less rod and dumbbell shape	16.3	0.81	70.1	-
F6	Spherical pellet	17.3	-	69.8	66.8
F7	Spherical pellet	16.4	-	72.3	72.4
F8	Spherical pellet	16.9	-	70.6	85.2
F9	Spherical pellet	17.8	-	68.5	71.1
F10	Spherical pellet	15.4	0.65	74.6	-
F11	Spherical pellet	15.6	0.69	71.2	-
F12	Hardness and unable to spheronization	18.7	1.12	65.3	-
F13	Hardness and unable to spheronization	21.3	1.41	47.6	-
F14	Spherical pellet	15.1	-	76.8	83.4
F15	Rod and dumbbell shape, hard wet mass	16.2	-	73.1	85.8
F16	Rod and dumbbell shape, hard wet mass	17.6	-	67.5	86.9

Note: the symbol of "-" expresses data not available.

formulation studies not only met the requirements of next process of fluid bed coating, but also needed to consider making drug release quickly and completely. The use of lactose and MCC as a filler can form sphere SDP with uniform size and appropriate friability through extrusion-spheronization. The addition of super disintegrant of CMS-Na ensured the rapid tacrolimus release in targeted site.

The characterization results of different tacrolimus-loading rate of 5%, 10%, 15%, 20% (F10, F11, F12, F13) are shown in the Table II. When the drug-loading rates were 15% and 20%, the wet masses were hard and difficult to be sieved to small pieces in extrusion process. However, when the drug-loading rate decreased to 10%, the quality of wet mass was significantly improved and smoothly passed through the sieve mesh on extruder. The SDP with low drug-loading rate was easily prepared but this may increase the oral dose. Therefore, tacrolimus-loading rate was determined to be 10%.

The PVP K30 was chosen as the binder agent using 30% ethanol solution as solvent. The proportion of the binder agent directly affected the viscosity of wet mass and pellet preparation. The optimal proportion of PVP K30 in formulation was determined by practical operation because the characteristics of binder agent were directly affected by the temperature and humidity in production environment. Silica gel was selected as glidant. Adhesion of lipophilic drug on the surface of silica gel contributed to accelerate the dissolution process. Talc flatted the cavity on the surface of pellets, which reduced the friction among the pellets in spheronization process. By the above screening tests of excipients, the composition and quantities of tacrolimus-SDP are listed in the Table III. It is worth noting that, as an intermediate of the formulation, tacrolimus-SDP showed a pretty high bioavailability compared to tacrolimus solution, which was demonstrated in subsequent pharmacokinetic experiment.

Preparation and *in vitro* release kinetic studies of tacrolimus colon-specific pellets

To perform film coating of tacrolimus-CSP, the tacrolimus-SDP was coated with mixed dispersion of Eudragit NE30D, Eudragit L30D55 and talc as time-dependent layer with specific weight gain in a mini lab-use fluidized bed coater. The weight gain was calculated as (final weight–original weight) / original weight × 100%. Next, the time-dependent layer coated pellets were further wrapped on the mixed dispersion of Eudragit L30D55, talc and triethyl citrate as pH-dependent layer. Eudragit

TABLE III - Composition of optimized tacrolimus-SDP

Ingredients	Quantities (%)	Functions
Tacrolimus solid dispersion	10	Active drug
Lactose and MCC (1:1.5, w:w)	83	Filler
CMS-Na	4	Disintegrate
Silica gel and talc	3	Lubricant and glidant
PVP K30	According to the practical operation	Binder

Note: MCC: microcrystalline cellulose; CMS-Na: carboxymethyl starch sodium; PVP: poly vinyl pyrrolidone.

NE30D is an acrylic coating material, with sustained released activity =and swelling in water (Liu *et al.*, 2013). Eudragit L30D55 is an anionic enteric-coating material, containing -COOH as a functional group and is selectively dissolved at pH > 5.5 solutions (He, Li, Tang, 2011). Talc is an anti-coherent agent to avoid the aggregation of pellets in the fluidized bed. The time-dependent layer consists of Eudragit NE30D and Eudragit L30D55. It is reported that the mixed Eudragit NE30D and Eudragit L30D55 coating can achieve controlled release of the drug and also help reduce the time-dependent layer deformation in the storage of the formulations (Wulff, Leopold, 2014).

The time-dependent coated pellets were prepared by different weight ratios of 2:1, 4:1, 8:1, 14:1, 16:1 (Eudragit NE30D: Eudragit L30D55) with a constant weight gain of 10%. Figure 1A shows their *in vitro* release in the sequential media of pH 1.2 HCl (0.1 mol/L), pH 6.8 and pH 7.2 PBS for 2 h, 3 h and the remaining 7 h. The cumulative release of coated pellets by different ratios of 2:1, 4:1, 8:1, 14:1, 16:1 was 48.36%, 37.98%, 31.46%, 23.94% and 20.11% in 0-5 h, respectively. At the same time point, the pellet with lower proportion of Eudragit

NE30D had a higher cumulative release level. The CSP should be stable in acid medium; almost no drug released in the pH 6.8 PBS and released most drugs in the pH 7.4 PBS. The pellets with the ratios of 14:1 and 16:1 had less cumulative releasein pH 6.8 PBS in 0-5 h, however, they did not release drug completely at 12 h. Therefore, the ratio of 8:1 (Eudragit NE30D: Eudragit L30D55) was selected for further studies.

A series of coated pellets were prepared by different thicknesses of time-dependent layer and quantified by the total weight gain (5%, 10%, 15%, 20%, 25%) difference with a constant Eudragit L30D55 to Eudragit NE30D ratio of 8:1. Figure 1B shows their cumulative release. The cumulative release of coated pellets was inversely proportional to the thicknesses of time-dependent layer. The cumulative release of 5% and 10% weight gain was 40.36% and 31.46% in 0-5 h., respectively. Tacrolimus was metabolized by cytochrome P450 3A in liver and intestine. CYP3A displayed significant interindividual and intraindividual variability (Hebert, 1997). Therefore, tacrolimus rapid release in a short time period may cause potential toxicological risk to liver and intestine due to its narrow therapeutic index. The cumulative release of 23.94% and 20.11% was obtained by the weight gain of 20% and 25% in 0-5 h, respectively; however, the drugs were not released completely in the time period of 0-12 h. Therefore, 15% weight gain was selected for time-dependent layer.

To achieve colon target release profile, the pH-dependent layer of Eudragit L30D55 was wrapped on the surface of time-dependent layer as an enteric coating. The formulation of interior time-dependent layer was constant according to the optimization. The 10% of triethyl citrate was used as plasticizer for Eudragit L30D55 in pH-dependent layer coating (Wagner *et al.*, 2000). Figure 1C shows the release profiles of the coated pellets with various weight gains (5%, 10%, 15%, 20%, 25%) of the pH-dependent layer in gradient pH solutions of
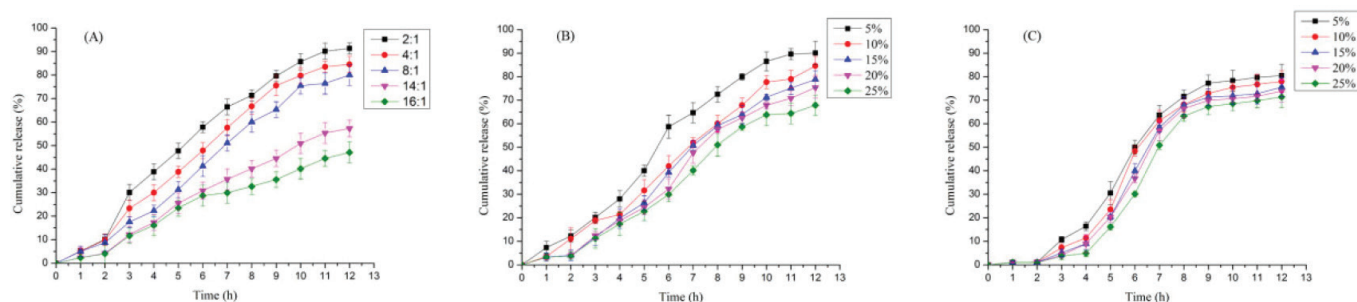

FIGURE 1 - The cumulative release of colon-specific pellets with (A) different ratios of Eudragit NE30D: Eudragit L30D55, (B) different weight gains of time-dependent layer, (C) different weight gains of pH-dependent layer. Data are presented as mean ± SD (n = 3).
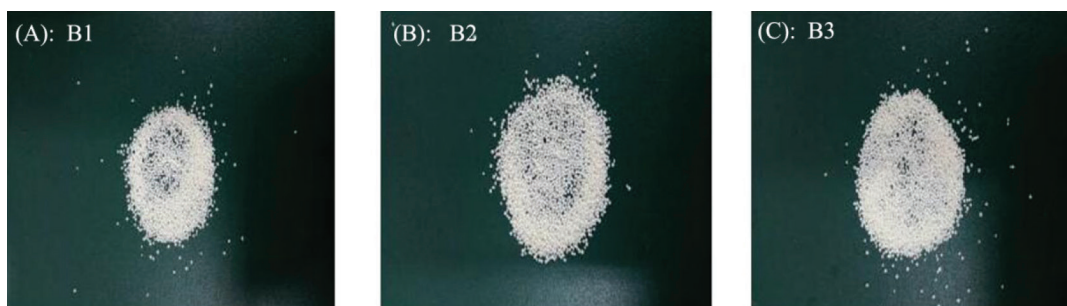
FIGURE 2 - Appearance of tacrolimus-CSP of optimized formulation. Batch numbers: (A) B1, (B) B2, (C) B3.

pH 1.2, pH 6.8 and pH 7.2 for 2 h, 3 h, and 7 h. Tacrolimus hardly released in the pH 1.2 HCl solution when the coating weight gain was greater than 5%. In pH 6.8 PBS, the pH-dependent layer of Eudragit L30D55 dissolved and exposed the internal time-dependent coated layer. Therefore, the initial release rate in the pH 6.8 PBS was determined by the thickness of pH-dependent layer. Taking into account the release rate of inner time-dependent layer, the pH-dependent layer of Eudragit L30D55 should not be too thick to release the inside drug. Therefore, 10% of weight gain was chosen as pH-dependent layer.

Three batches of tacrolimus-CSP (B1, B2, B3) were prepared according to the final optimized time- and pH-dependent layer formulations. The appearance of optimized tacrolimus-CSP is shown in Figure 2. Figure 3 shows thier *in vitro* cumulative release. The release curve showed that tacrolimus was hardly detected in pH 1.2 HCl solution in initial 2 h. This suggested that pH-dependent layer could protect CSP from disintegration in the gastric acid. In pH 6.8 PBS, the pH-dependent layer was gradually dissolved and the interior time-dependent layer performed a sustained-release function. Therefore, tacrolimus released from CSP slowly at 2-5 h. Furthermore, tacrolimus-CSP performed a quick and complete drug release in the pH 7.2 stimulated colonic juice. The cumulative release profiles showed that curve raised sharply after 5 h and still had a release trend after 12 h. This mainly owed to the super disintegrating agent of CMS-Na and time-dependent coating of Eudragit NE30D in CSP. The encapsulation efficiency of tacrolimus-CSP was 89.88% and the drug-loading rate was 1.499%.

According to the fit curve results of tacrolimu-CSP dissolution, it appeared that the Weibull model was the most appropriate to its *in vitro* release. The correlation coefficient (R^2) and model selection criterion (MSC) were 0.9908 and 3.3996, respectively. When comparing different models, the most appropriate model appeared the largest MSC. Generally, the value of MSC higher than two to three suggested a good fit (Mayer *et al*., 1999). The
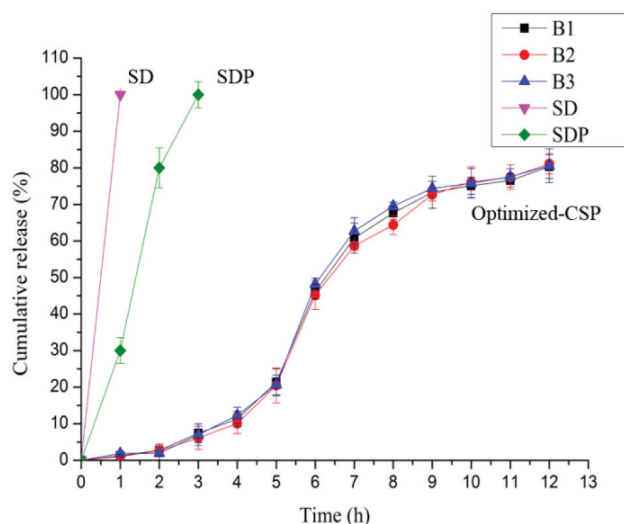

FIGURE 3 - The cumulative release of tacrolimus-SD, tacrolimus-SDP and tacrolimus-CSP of optimized formulations. Data are presented as mean ± SD (n = 3). Batch numbers of tacrolimus-CSP: (■) B1, (●) B2, (▲) B3.

values of initial parameter (α, β, Ti) of Weibull equation were calculated and given in Table IV. The *n* value of Koresmeyer–Peppas model was 1.243, indicating tacrolimus release from the CSP consistent with supercase-II transport in the mechanism of matrix dissolution. The MDT value of optimized tacrolimus-CSP was found to be 5.98. The result indicated that tacrolimus mainly released in the pH 7.2 stimulated colonic juice, which agreed with the *in vivo* sustained-release profile of MRT. Time in hours to take 10% and 80% drug release can predict the ability of colon-specific release from CSP. The T10% and T80% values of optimized CSP were found to be 3.149 h and 10.655 h respectively. From these parameters, tacrolimus-CSP prospectively showed 2-3 h lag time, which provided most or complete drug release in colon. All these results are given in Table IV. Furthermore, from the cumulative release curve in Figure 3, the release behavior of CSP was significantly different with SD and SDP. The value of f_2 between dissolution profiles of CSP and SDP was found to be 13.54.

TABLE IV - Release kinetics of optimized tacrolimus-CSP

	Weibull equation								
	α	β	Ti	MSC	R²	n	MDT	T10%	T80%
Tacrolimus-CSP	5.714	1.082	3.141	3.3996	0.9908	1.243	5.98	3.149	10.655

Note: *MDT*: mean dissolution time; MSC: model selection criterion; *n*: diffusional exponent; R²: correlation coefficient; T10%: time to release 10% drug; T80%: time to release 80% drug; *Ti*: the location parameter which represents the lag time before the onset of the dissolution or release process; *α*: the scale parameter which defines the time scale of the process; *β*: the shape parameter which characterizes the curve, S-shaped with upward curvature followed by a turning point ($\beta > 1$), or parabolic with a higher initial slope and after that consistent with the exponential ($\beta < 1$) (Zhang *et al.*, 2010).

In vivo pharmacokinetics studies

The representative UPLC chromatograms of blank blood sample, blank blood sample spiked with tacrolimus and ascomycin, the blood sample after oral administration of tacrolimus-CSP are shown in Figure 4. The chromatograms showed a stable base line, good resolution between tacrolimus and ascomycin and no other endogenous interference at the retention time of tacrolimus. The concentration of tacrolimus was determined by standard calibration curve in a range of 20.37-1001.8 ng/mL with a reference substance. The regression equation was $A = 3.016 \times 10^{-4}C - 2.831 \times 10^{-4}$

($r = 0.9992$). The mean absolute recovery and the mean relative recovery were $71.31 \pm 4.47\%$ and $94.84 \pm 1.44\%$, respectively. The lower detection limit of this method was 20 ng/mL. The RSD for intra-day and inter-day precision were 5.85% and 2.92%, respectively, indicating good reproducibility. In summary, this internal standard method demonstrated acceptable accuracy, precision, and linearity, and was used to determine tacrolimus concentration in rat blood.

To investigate the absorption enhancement of tacrolimus-SDP and tacrolimus-CSP, a total of of 18 rats were chosen for the pharmacokinetic experiment with the tacrolimus suspension as a control. The blood tacrolimus
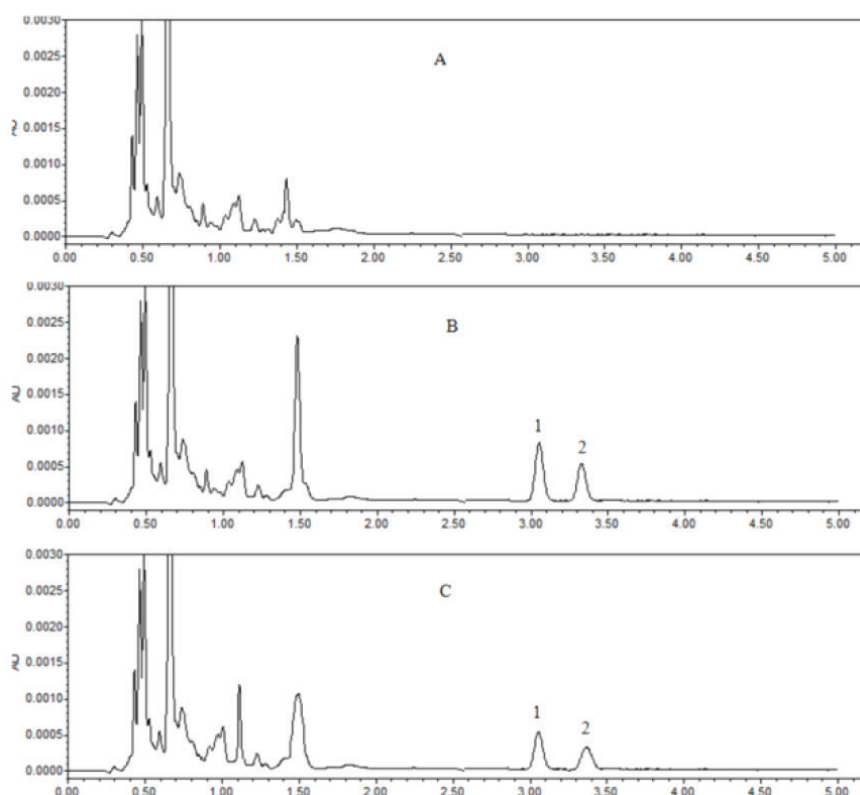

FIGURE 4 - UPLC chromatograms of (A) blank blood sample, (B) blank blood sample spiked with tacrolimus and ascomycin, (C) the blood sample after oral administration of tacrolimus-CSP. 1: ascomycin; 2: tacrolimus.

concentration-time curves are shown in Figure 5. The blood tacrolimus concentration from tacrolimus-SDP group was obviously higher than tacrolimus suspension group. The reason was that the SD technology increased the solubility of tacrolimus and enhanced its absorption in intestine part. Tacrolimus was not detected in first 1 h after administration of the tacrolimus-CSP group. After 4.5 h, the blood concentration of the tacrolimus-CSP group began to gradually drop and maintained a steady state for a long time. The time to achieve maximum blood concentration of the CSP group was longer than other two groups. This implied that CSP had a less drug release in stomach and upper small intestine. The $MRT_{(0-\infty)}$ value of tacrolimus-CSP group was 1.87-fold longer than that of the tacrolimus-SDP group (15.57 ± 3.04 *vs.* 8.32 ± 5.96), suggesting CSP had a good ability to maintain a stable
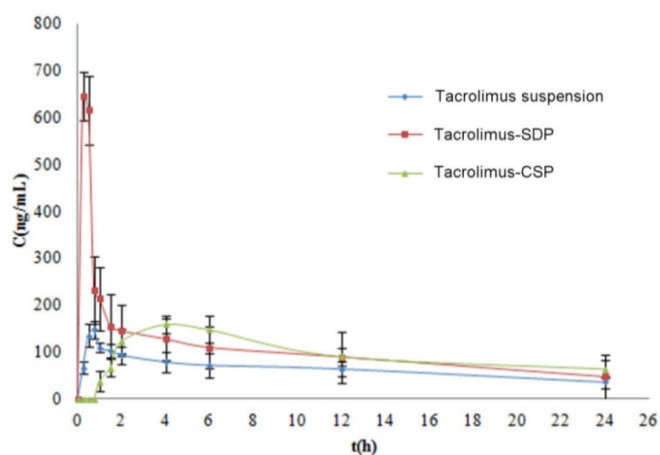

FIGURE 5 - Blood concentration–time curves of tacrolimus in rats. ◆:The blood tacrolimus concentration from tacrolimus suspension group. ■: The blood tacrolimus concentration from tacrolimus-SDP group. ▲: The blood tacrolimus concentration from tacrolimus-CSP group. Data are presented as mean ± SD (n = 6).

blood concentration state and a potential developed into sustained-release formulations.

Some important pharmacokinetic parameters were calculated using DAS 2.0 pharmacokinetics software and are shown in Table V. The best compartment models of the three groups in rats were both two-compartment models with a weight coefficient of 1. The $AUC_{(0-\infty)}$ and Cmax value of tacrolimus-CSP were 1.99-fold and 4.39-fold higher than that obtained after administration of tacrolimus suspension (1568.16 ± 214.97 *vs.* 1090.23 ± 125.53 ng·h/mL), (195.68 ± 3.14 *vs.* 147.51 ± 5.34 ng/mL), respectively, which clearly confirmed the role of CSP in enhancing the bioavailability of tacrolimus. The underlying reason was that tacrolimus co-precipitating with HPMC, a hydrophilic excipient, formed an amorphous state in the pellets, which increased the solubility of tacrolimus in the colon juice. In addition, CMS-Na facilitated water penetration into the pellet core which led to a faster drug release. Given the BCS class II drug of tacrolimus, the dissolution rate was the rate-limiting step for its *in vivo* absorption (Wang, Gan, Zhang, 2011). Therefore, the tacrolimus in CSP was more easily absorbed compared to tacrolimus suspension through oral administration. However, the $AUC_{(0-\infty)}$ and Cmax values of tacrolimus-CSP group were lower than that obtained by administration of the tacrolimus-SDP group (1568.16 ± 214.97 *vs.* 2479.58 ± 183.33 ng·h/mL, 195.68 ± 3.14 *vs.* 646.16 ± 8.15 ng/mL). It suggested that the drug concentration absorbed into the blood circulation was less than that in the colon tissue, which was conducive to the increase of drug concentration in colon. Prolonged MRT, reduced Cmax and trending toward a delayed Tmax were observed for CSP. These results clearly indicated that the controllable release of tacrolimus from CSP successfully resulted in the targeted absorption *in vivo*.

TABLE V - Pharmacokinetic parameters of tacrolimus suspension, tacrolimus-SDP, tacrolimus-CSP groups after oral administration to rats

Parameter	Tacrolimus suspension	Tacrolimus-SDP	Tacrolimus-CSP
$t_{1/2\alpha}$(h)	1.23 ± 0.02	2.07 ± 0.55	23.42 ± 0.81*
$t_{1/2\beta}$(h)	11.69 ± 1.65	27.63 ± 2.04	69.31 ± 4.91*
Tmax (h)	0.75 ± 0.02	0.5 ± 0.03	4.5 ± 0.24**
Cmax (ng/mL)	147.51 ± 5.34	646.16 ± 8.15	195.68 ± 3.14**
$AUC_{(0-\infty)}$(ng·h/mL)	1090.23 ± 125.53	2479.58 ± 183.33	1568.16 ± 214.97*
$MRT_{(0-\infty)}$(h)	5.14 ± 3.24	8.32 ± 5.96	15.57 ± 3.04*

Note: Data are presented as mean ± SD (n = 6). $t_{1/2}$: biological half-life; Cmax: maximum blood concentration; Tmax: peak time; AUC: area under the curve; MRT: mean residence time. *$p < 0.05$ vs. tacrolimus suspension group; **$p < 0.01$ vs. tacrolimus suspension group

In vitro-in vivo correlation

IVIVC was carried out for optimized tacrolimus-CSP by plotting the *in vitro* cumulative percentage of tacrolimus release on X-axis and the cumulative AUC obtained after oral administration on Y-axis. From the Figure 6, the least square regressions yielded an essentially linear pattern ($R^2 > 0.95$), indicating a good correlation between the *in vitro* cumulative percentage of drug release and *in vivo* drug absorption.
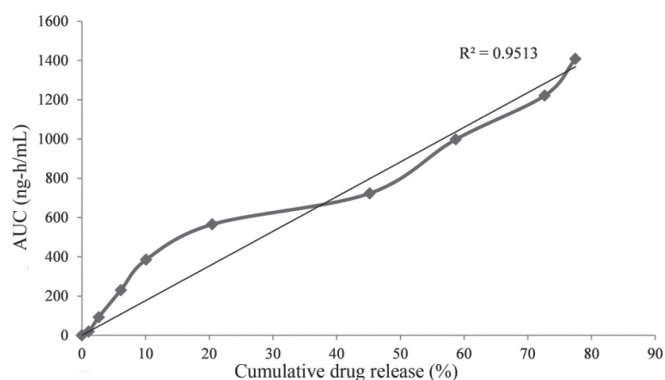

FIGURE 6 - *In vitro-in vivo* correlation plot of optimized tacrolimus-CSP.

In vivo biodistribution studies

Figure 7 shows the biodistribution of tacrolimus after oral administration of suspensions, SDP and CSP examined based on µg tacrolimus per g tissue. At 2-6 h after oral administration of tacrolimus-suspensions and tacrolimus–SDP, a fast and wide distribution of drug was observed in stomach, proximal and distal small intestine, cecum and colon. However, tacrolimus derived from CSP was nearly no drug detected in the stomach and proximal small intestine, which indicated a control-release effect in gastrointestinal tract. Tacrolimus derived from CSP was mostly accumulated in cecum and colon at 6-24 h.
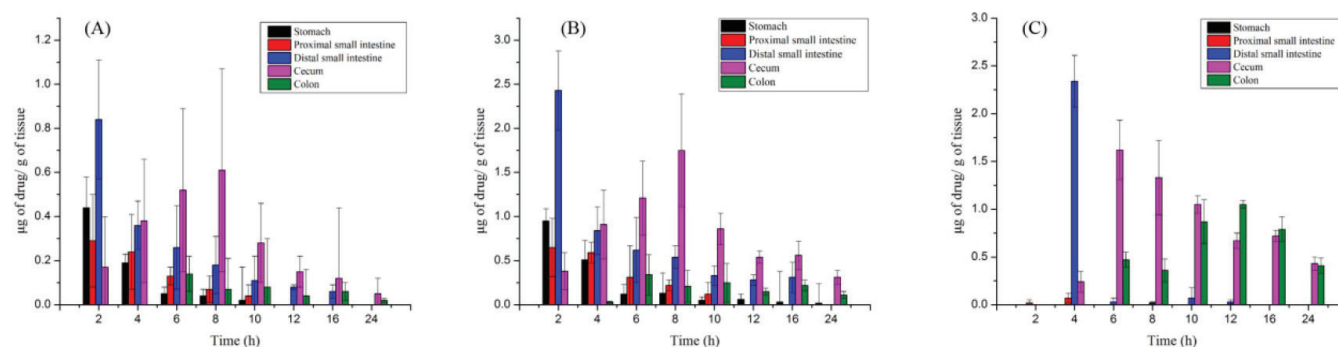
Twelve hours after oral administration, tacrolimus showed a remarkable selective distribution in colon, about 1.57-fold to cecum and 35-fold to distal small intestine. Yura *et al.* (1999) evaluated the distribution of radioactive [³H]-labelled tacrolimus solution in rat by intravenous administration. They confirmed that 64% dose of [³H]-tacrolimus was accumulated in the small intestine after 8 h following injection (Yura *et al.*, 1999). Once in bloodstream, tacrolimus was inclined to distribute into fatty organs like liver and small intestine due to its highly lipophilic nature. Therefore, tacrolimus loaded by colon-specific delivery system and selectivity release drug in the colon part might be favorable for therapeutic efficacy enhancement for colitis. Furthermore, in the end of time period, tacrolimus concentration from CSP group was higher than the tacrolimus-suspension and tacrolimus–SDP group, suggesting that the absorption and elimination of drug derived from CSP maybe occur at the same time in colon. However, in other two groups, there were only elimination processes in the end of time period. These results proved that the combination of OCDDS and SD technology successfully improved the solubility of tacrolimus in the colon juice and facilitated the absorption for oral administration. In general, the order of tacrolimus accumulation in the colon was as follows: CSP > SDP > suspensions.

It is worth noting that toxicity of tacrolimus is dose dependent, and this was confirmed in a retrospective study of patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease treated with low dose tacrolimus. This research indicated that lower target serum concentration levels of tacrolimus may reduce the incidence of adverse events (Baumgart *et al.*, 2006). Colon targeted tacrolimus-CSP may reduce the potential systemic toxicity of immunosuppressant, such as tremor, headache, diarrhea, hypertension and nausea, compared to clinical used tacrolimus capsules or injections (Baumgart *et al.*, 2008). A further consideration of tacrolimus-CSP should

FIGURE 7 - Tacrolimus concentrations in different tissues after oral administration of (A) tacrolimus suspension, (B) tacrolimus-SDP, (C) tacrolimus-CSP. Data are presented as mean ± SD (n = 3).

be given to the increased risk of intestinal side reaction and enterotoxicity due to the rapid release effect of SD in colon

CONCLUSION

A novel OCDDS with SD technology was successfully developed in this study. Tacrolimus-CSP was prepared by tacrolimus-SDP using extrusion-spheronization and coated with pH-dependent layer (Eudragit L30D55) and time-dependent layer (Eudragit L30D55 and Eudragit NE30D). Based on *in vitro* drug release studies, optimized tacrolimus-CSP showed a significant drug release level in the pH 7.4 PBS. The drug release from optimized tacrolimus-CSP followed Weibull model and the drug release mechanism followed supercase-II transport. *In vivo* pharmacokinetic and biodistribution studies indicated that the tacrolimus-CSP negligibly released the drug in stomach and small intestine, but specifically delivered the drug to colon and showed a slow and sustained drug absorption compared to tacrolimus-SDP and tacrolimus suspensions. From the IVIVC results, there was a good correlation between the *in vitro* and *in vivo* parameters. The local immunosuppression effects of tacrolimus may decrease unwanted gastrointestinal irritation and systemic toxicity compared to conventional injection administration. In conclusion, it seems that the combined SD pellets with both pH-sensitive and time-dependent coating methods would be a promising approach for colon targeted delivery of tacrolimus.

ACKNOWLEDGMENTS

DECLARATION OF INTEREST

The authors report no conflicts of interest in this work.

REFERENCES

Bansal V, Malviya R, Malaviya T, Sharma PK. Novel prospective in colon specific drug delivery system. Polim Med. 2014;44(2):109-118.

Baumgart DC, Pintoffl JP, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease--a long-term follow-up. Am J Gastroenterol. 2006;101(5):1048-56.

Baumgart DC, MacDonald JK, Feagan B. Tacrolimus (FK506) for induction of remission in refractory ulcerative colitis. Cochrane Database Syst Rev. 2008;16(3):CD007216.

Bose A, Elyagoby A, Wong TW. Oral 5-fluorouracil colon-specific delivery through in vivo, pellet coating for colon cancer and aberrant crypt foci treatment. Int J Pharm. 2014;468(1-2):178.

De Toni L, Di Nisio A, Magagna S, Michielan A, Martinato M, Sturniolo GC, et al. Altered chemokine signalling in endothelial progenitor cells from acute ulcerative colitis patients. Gastroenterol Res Pract. 2015;2015:843980.

Dhandapani NV. Pelletization by extrusion-spheronization-a detailed review. Optical Engineering. 2005;44:125202.

Dodoo C, Wang J, Basit AW, Stapleton P, Gaisford S. Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation. Int J Pharm. 2017;530(1-2):224.

Fang H, Ma P, Gui S. The new choice of tacrolimus in the treatment of refractory ulcerative colitis. MOJ Bioequivalence Bioavailability. 2015;1(3):00011.

Fang H, Guo J, Gui S, Niu M. Preparation and in vitro dissolution of tacrolimus solid dispersion. Clin Hosp Pharm J. 2016;36:710-714.

Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. Adv Drug Deliv Rev. 1997;27(2-3):201-214.

He H, Li H, Tang X. Preparation of pH-dependent modified-release pellets of urapidil to improve its bioavailability. Pharm Dev Technol. 2011;16(3):212-218.

Landy J, Wahed M, Peake ST, Hussein M, Ng SC, Lindsay JO, et al. Oral tacrolimus as maintenance therapy for refractory ulcerative colitis--an analysis of outcomes in two London tertiary centres. J Crohns Colitis. 2013;7(11):e516-521.

Liu Q, Gong Y, Shi Y, Jiang L, Zheng C, Ge L, et al. A novel multi-unit tablet for treating circadian rhythm diseases. AAPS PharmSciTech. 2013;14(2):861-869.

Mayer BX, Mensik C, Krishnaswami S, Derendorf H, Eichler HG, Schmetterer L, et al. Pharmacokinetic-pharmacodynamic profile of systemic nitric oxide-synthase inhibition with L-NMMA in humans. Br J Clin Pharmacol. 1999;47(5):539-544.

Miyoshi J, Matsuoka K, Inoue N, Hisamatsu T, Ichikawa R, Yajima T, et al. Mucosal healing with oral tacrolimus is associated with favorable medium- and long-term prognosis in steroid-refractory/dependent ulcerative colitis patients. J Crohns Colitis. 2013;7(12):e609-614.

Navas-Lopez VM, Blasco Alonso J, Serrano Nieto MJ, Giron Fernandez-Crehuet F, Argos Rodriguez MD, Sierra Salinas C. Oral tacrolimus for pediatric steroid-resistant ulcerative colitis. J Crohns Colitis. 2014;8(1):64-69.

Ogata H, Matsui T, Nakamura M, Iida M, Takazoe M, Suzuki Y, et al. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. Gut. 2006;55(9):1255-1262.

Park H J, Jung H J, Ho M J, Lee DR, Cho HR, Choi YS, et al. Colon-targeted delivery of solubilized bisacodyl by doubly enteric-coated multiple-unit tablet. Eur J Pharm Sci. 2017;102:172-179.

Park SC, Jeen YT. Current and emerging biologics for ulcerative colitis. Gut Liver. 2015;9(1):18-27.

Schellekens RC, Stuurman FE, van der Weert FH, Kosterink JG, Frijlink HW. A novel dissolution method relevant to intestinal release behaviour and its application in the evaluation of modified release mesalazine products. Eur J Pharm Sci. 2007;30(1):15-20.

Tajdaran K, Shoichet MS, Gordon T.Borschel GH. A novel polymeric drug delivery system for localized and sustained release of tacrolimus (FK506). Biotechnol Bioeng. 2015;112(9):1948-1953.

Tsunashima D, Yamashita K, Ogawara K, Sako K, Higaki K. Preparation of extended release solid dispersion formulations of tacrolimus using ethylcellulose and hydroxypropylmethylcellulose by solvent evaporation method. J Pharm Pharmacol. 2016;68(3):316-323.

Veerareddy PR, Vemula SK. Formulation, evaluation and pharmacokinetics of colon targeted pulsatile system of flurbiprofen. J Drug Target. 2012;20(8):703-14.

Vemula SK. Colon specific drug delivery: effect of Eudragit enteric coating on hydroxypropyl methylcellulose matrix tablets of flurbiprofen. J Young Pharm. 2015a;7(4):373-383.

Vemula SK. Formulation and pharmacokinetics of colon-specific double-compression coated mini-tablets: Chronopharmaceutical delivery of ketorolac tromethamine. Int J Pharm. 2015b;491(1-2):35-41.

Vemula SK. A novel approach to flurbiprofen pulsatile colonic release: formulation and pharmacokinetics of double-compression-coated mini-tablets. Aaps Pharmscitech. 2015c;16(6):1465-73.

Vemula SK, Veerareddy PR, Devadasu VR. Pharmacokinetics of colon-specific pH and time-dependent flurbiprofen tablets. Eur J Drug Metab Pharmacokinet. 2015;40(3):301-311.

Vemula SK, Veerareddy PR. Development, evaluation and pharmacokinetics of time-dependent ketorolac tromethamine tablets. Expert Opin Drug Deliv. 2013;10(1):33-45.

Wagner KG, Krumme M, Beckert TE, Schmidt PC. Development of disintegrating multiple-unit tablets on a high-speed rotary tablet press. Eur J Pharm Biopharm. 2000;50(2):285-292.

Wang Y, Gan Y, Zhang X. Novel gastroretentive sustained-release tablet of tacrolimus based on self-microemulsifying mixture: in vitro evaluation and in vivo bioavailability test. Acta Pharmacol Sin. 2011;32(10):1294-302.

Wulff R, Leopold CS. Coatings from blends of Eudragit(R) RL and L55: a novel approach in pH-controlled drug release. Int J Pharm. 2014;476(1-2):78-87.

Xu M, Sun M, Qiao H, Ping Q, Elamin ES. Preparation and evaluation of colon adhesive pellets of 5-aminosalicylic acid. Int J Pharm. 2014;468(1-2):165-171.

Yamashita K, Nakate T, Okimoto K, Ohike A, Tokunaga Y, Ibuki R, et al. Establishment of new preparation method for solid dispersion formulation of tacrolimus. Int J Pharm. 2003;267(1-2):79-91.

Yura H, Yoshimura N, Hamashima T, Akamatsu K, Nishikawa M, Takakura Y, et al. Synthesis and pharmacokinetics of a novel macromolecular prodrug of Tacrolimus (FK506), FK506-dextran conjugate. J Control Release. 1999;57(1):87-99.

Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, et al. DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. AAPS J. 2010;12(3):263-271.