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RESEARCH ARTICLE

Enhanced oral bioavailability of tacrolimus in rats by self-microemulsifying drug delivery systems

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Abstract

A new self-microemulsifying drug delivery system (SMEDDS) has been developed to increase the solubility, dissolution rate and oral bioavailability of tacrolimus (TAC). The formulations of TAC-SMEDDS were optimized by solubility assay, compatibility tests, and pseudo-ternary phase diagrams analysis. In order to inhibit the efflux of P-glycoprotein (P-gp) for tacrolimus, which is the substrate of P-gp, the excipients which show the inhibition effect to P-gp, such as tocopheryl polyethylene glycol succinate (TPGS) and Cremophor EL40, were chosen in the SMEDDS formulations. According to particle size and the rate of self-emulsification, two optimized formulations were selected: Miglyol 840 as oil phase, Transcutol P as cosurfactant, TPGS as surfactant (TPGS-SMEDDS) or Cremophor EL40 as surfactant (Crem-SMEDDS), respectively. The ratio of oil phase, surfactant and cosurfactant is 1:7.2:1.8. The mean droplet size distribution of the optimized SMEDDS was less than 20 nm. The *in vitro* dissolution test indicated a significant improvement in release characteristics of TAC. The prepared SMEDDS was compared with the homemade solution by administering the hard capsule to fasted rats. The absorption of TAC from TPGS-SMEDDS and Crem-SMEDDS form resulted in about sevenfold and eightfold increase in bioavailability compared with the homemade solution. Our study illustrated the potential use of SMEDDS for the delivery of hydrophobic compounds, such as TAC by the oral route.

Keywords: SMEDDS, tacrolimus, P-glycoprotein, TPGS, Cremophor

Introduction

Tacrolimus (TAC) is an immunosuppressive drug that is mainly used after allogeneic organ transplant to reduce the activity of the patient's immune system and so lower the risk of organ rejection[1–3]. TAC is currently available in both intravenous and oral dosage form (commercially known as Prograf®). The oral pharmacokinetics of these agents is highly variable. After oral administration, TAC exhibits low and variable bioavailability ranging from 4% to 89%, with a mean of ~25%, in liver and kidney transplant patients[4,5]. Some causes are responsible for the large heterogeneity: (i) poor water-solubility, (ii) extensively metabolized from cytochrome P450 3A4 (CYP3A4) metabolism, (iii) P-glycoprotein (P-gp) efflux transport within the intestinal epithelium. Several approaches such as multiple emulsion[6], liposomes[7], cyclodextrins[8], solid dispersion formulation[9], microsphere[10], nanocapsules[11], and nanopartilces[12] have been investigated to improve oral delivery or local delivery of TAC.

This study focused on investigating a new self-microemulsifying drug delivery system (SMEDDS) of TAC in order to overcome the above-mentioned problems to improve bioavailability. SMEDDS is defined as an isotropic mixture of lipid, surfactant, cosurfactant and drug substance that rapidly form a microemulsion upon mixing with water. The surfactants act by dispersing the lipid formulation in the gastrointestinal tract upon dilution with the gastrointestinal fluid. This results in the formulation of fine droplets providing a large surface area and thereby promoting a rapid release of TAC. Enhancement of drug dissolution of TAC could be achieved by use of SMEDDS. Moreover, the lipid components of SMEDDS could promote the intestinal lymphatic transport of drugs due to lipoprotein/chylomicron production[13–15].

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Recently, some excipients, such as D-α-tocopheryl polyethylene glycol succinate 1000 (TPGS) and Cremophor, can inhibit both pre-systemic drug metabolism and intestinal efflux mediated by P-gp[16–18]. Thus, TPGS and Cremophor were present in the developed SMEDDS formulations. Bioavailability of orally administered TAC-SMEDDS was evaluated in rats by comparison with the homemade solution.

Materials and methods

Materials

TAC was purchased from Chengdu Chuankang Wanle Pharmceutical Co., Ltd. Cremophor EL40 was a gift from BASF Corp. (Mount Olive, NJ). Transcutol P and Maisine35-1 were kindly supplied by Gattefosse Co. (Shanghai, China). Castor oil was purchased from Shanghai Baorui Chemical Co., Ltd. (Shanghai, China). Miglyol 840 was from Condea Chemie GmbH (Witten, Germany). PEG 200 and 1,2-propylene glycol were from Beijing Yili Fine Chemical Co. (Beijing, China). TPGS was purchased form Wuhan Yuancheng Technology Development Co., Ltd. (Wuhan, China). Hard gelatin capsules were obtained as gift samples from Guangsheng Capsules Co. (Shanxi, China). Double-distilled water was used. Acetonitrile was of high-performance liquid chromatography (HPLC) grade (Fisher Company, Fair Lawn, NJ). All other reagents were of analytical grade.

Solubility of TAC

Solubility of TAC in various oils, surfactants, and cosurfactants was measured by shake flask method. An excess amount of TAC was introduced into 2 mL of each vehicle, and mixture was kept in sealed vials. The mixture was then kept at 37°C in an air oscillator for 72 h to reach equilibrium. The equilibrated sample was centrifuged at 5000 rpm for 10 min using a centrifuge (TGL-16B, Shanghai Anting Scientific Instrument Factory, Shanghai, China). Undissolved TAC was removed by filtering with a membrane filter (0.45 μm). The concentration of TAC in the supernatants was determined by HPLC analysis.

HPLC analysis of TAC

The solubility of TAC in various vehicles was determined by a validated reverse-phase HPLC method. The HPLC apparatus consisted of L-2130 HPLC pump (Hitachi, Tokyo, Japan) equipped with a L-2420 UV detector (Hitachi). TAC was separated on a RP-C_{18} column (Welch ODS C_{18}, 5 μm, 200 × 4.6 mm) using acetonitrile-0.03 M monoammonium phosphate (65:35) as mobile phase at a flow rate of 1.0 mL·min^{-1} with the detection wavelength at 230 nm. The HPLC method was linear (r = 0.999) in the concentration range of 10.0–200.0 μg·mL^{-1}. The method was validated with respect to accuracy, inter-day and intra-day precision, stability, and reproducibility as per International Conference on Harmonisation guidelines and the relative standard deviation was less than 1.8% in all cases.

Preparation of SMEDDS formulation

The TAC contents in the marketed formulations are 5 mg. In the SMEDDS, the content of TAC was fixed constant (0.8% w/w of the vehicle). Components of SMEDDS (oil, surfactant, cosurfactant, and TAC) were accurately weighed into glass beaker and heated at 35°C in an air oscillator until TAC dissolved. At the optimized formulation, the fill volume of a size 0 capsule containing 5 mg TAC was used for dissolution and bioavailability studies.

Pseudo-ternary phase diagram study

Pseudo-ternary phase diagrams were constructed by progressive titration of the component mixtures in the presence of the drug. Phase behavior of systems was studied at various ratios of surfactant to cosurfactant (K_{m1}) viz. 4:1, 3:1, 2:1, 1:1 and 1:2. Mixtures of surfactant and cosurfactant (at a specific K_{m2}) with oil were prepared at ratios 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9. Each mixture was then titrated by adding water up to clouding. After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired components ratios.

Emulsion droplet size analysis

200 μl SMEDDS was diluted with 20 mL water in a glass flask and gently stirred. A Nicomp™ 380 ZLS Zeta Potential/Particle sizer (PSS Nicop, Santa Barbara, CA) was utilized to determine the droplet size and distribution. The particle sizes range that the analyzer could measure was from 2 nm to 5000 nm. Each sample was analyzed in triplicate.

In vitro dissolution study

Dissolution tests were performed with a dissolution apparatus (Rc2-6B, Tianjin University Electronics Co., Ltd., Tianjin, China). The dissolution medium was 250 mL of 0.5% Tween20 pH6.8 phosphate buffer, under the rotation speed of 100 rpm at 37 ± 0.5°C. To determine the amount of TAC dissolution from the test preparations, 1.0 mL sample was withdrawn at 5, 10, 20, 30, 45, 60, 90, 120, and 180 min and then an equal volume of temperature equilibrated blank media was added into the beaker. Concentration of TAC was analyzed by HPLC system. Each release test was carried out in triplicate.

In vivo study

The bioavailability experiment was designed to compare SMEDDS and TAC homemade solution. TAC homemade solution was prepared according to the following methods: PEG 400, ethanol and purified water was mixed together according to the ratio of 1:1:3. The desired amount of TAC was then added to the mixture obtained above and mixed. *In vivo* study was approved by the Animal Ethics Committee of Shenyang Northern Hospital. The male Wister rats (220–250 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). They were allocated to three groups at random. The animals were

fasted for 12 h prior to the oral administration of TPGS-SMEDDS, Crem-SMEDDS and homemade solution with a dose of 10 mg/kg. After dosing for 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720 and 1440 min, 400 μL blood samples were obtained from the retro-orbital plexus with a heparinized tube. Blood samples were stored at –20°C until analysis.

Instrumentation and UPLC-MS-MS conditions

Analyses were acquired on an ACQUITY UPLC™ system (Waters Co.,). An ACQUITY UPLC™ BEH C18 column (50 mm × 2.1 mm, 1.7 μm) was used. The chromatographic separations were accomplished using gradient elution with a mobile phase composed of acetonitrile and water containing 0.1% formic acid. The gradient elution program in the initial stage was maintained at 15% water for 0.2 min, changed linearly to 10% water (0.2–0.5 min), then changed linearly to 5% water (0.5–2.8 min) followed by a return to the initial conditions. The column temperature was maintained at 50°C with the flow rate set at 0.2 mL/min. The injection volume was 10 μL using the partial loop mode for sample injection.

The separated compounds were detected by a Waters Tandem Quadrupole (TQ) Detector (Waters Co.). The mass spectrometer was operated with an electrospray ionization interface in positive ionization mode. The ionization source conditions were: capillary voltage of 3.2 kV, cone voltage of 50 V, the optimized collision energy was 34 V and 19 V for TAC and glibenclamide, respectively; source temperature 120°C and desolvation temperature 350°C. The cone and desolvation gas flow rates were 50 L/h and 500 L/h, respectively, and were obtained from an in-house nitrogen source, Argon was used as collision gas. Under these UPLC-MS-MS conditions, the compounds were analyzed by multiple reaction monitoring of the transitions of m/z 826.3→616.5 for TAC and m/z 494.0→352.0 for glibenclamide (I.S.), respectively. The scan time was set at 0.02 s per transition. Data were acquired using Masslynx 4.1 software.

Sample preparation

Methylene dichloride and n-hexane (1:1) mixture (2 mL), 100 μL of I.S. solution (20 μg/mL) were added to the 100 μL blood sample. After vortex-mixing for 3 min and centrifugation at 3500 rpm for 10 min, the upper organic layer was transferred and evaporated to dryness at 35°C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL mobile phase followed by vortex-mixing and centrifugation at 15000 rpm for 10 min. Then, 10 μL of an aliquot of supernatant was injected for analysis.

Pharmacokinetic study

The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed values from the experimental data. The area under the plasma concentration–time curve from zero to the time of the final measurable sample (AUC_{0-t}) was calculated using the linear-trapezoidal rule up to the last sampling point with the detectable level (C). The area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the linear-trapezoidal rule with extrapolation to infinity.

Statistical evaluation was performed by Student *t*-test of the paired observations to analyze the different concentrations of TAC. $p < 0.05$ were considered to indicate significant differences. Datas were expressed as mean ± SD.

Results and discussion

Solubility studies

The self-emulsifying formulations consisted of oil, surfactants, cosurfactants, and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution. The solubilities of TAC in various vehicles were presented in Table 1. Note that TPGS and Cremophor were fixed to be as surfactant due to its inhibiton to metabolism and efflux. Miglyol 840 and Transcutol provided higher solubility than other vehicles, then Miglyol 840 and Transcutol were chosen as oil and cosurfactant, respectively.

Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed to identify the self-emulsifying regions and to optimize the concentration of oil, surfactant and cosurfactant. The series of SMEDDS were prepared and their self-emulsifying properties were visually observed[19]. The phase diagrams of the SMEDDS containing TPGS, Miglyol 840 and Transcutol were shown in Figure 1. The phase diagrams containing Cremophor EL40, Miglyol 840 and Transcutol were shown in Figure 2. As shown in Figures 1 and 2, all the diagrams possessed a broad microemulsion area, no matter what was employed as the surfactant. Microemulsion formation area was increased with an increase in K_{m1} and K_{m2}. Hence, K_{m1} and K_{m2} were maintained at 4:1 and 9:1, respectively. Based on the results, SMEDDS formulation was established: 10% Miglyol 840 as oil, 72% TPGS or Cremophor EL40 as surfactant and 18% Transcutol as cosurfactant.

Particle size analysis

SMEDDS concentrated solution with 0.8% TAC was diluted 100-fold with distilled water, then the size

Table 1. The saturated solubility of tacrolimus in different vehicles (mg/ mL, 35°C).

Vehicle	Solubility	Vehicle	Solubility
Miglyol 840	17.93	Cremophor EL40	5.44
Maisine35-1	2.47	Transcutol P	359.42
Castor oil	4.31	PEG 200	76.89
TPGS	28.29	1,2-propanediol	66.95

distribution was determined. Typical size distribution was shown in Figure 3. It was observed that the droplet size distribution of TPGS-SMEDDS formulation with TAC (17.7 ± 3.5) was much the same as that of Crem-SMEDDS formulation with TAC (17.9 ± 4.6).

In order to simulate *in vivo* dilution behavior of SMEDDS, effect of dilution media including distilled water, 0.1 M hydrochloride solution and pH 6.8 phosphate buffer was evaluated. The results showed that the dilution media had little effect on particle size and time of self-microemulsifying.

In vitro dissolution study

In order to keep sink condition, 0.5% Tween20 pH6.8 phosphate buffer was chosen as dissolution media. *In vitro* dissolution profile of TPGS-SMEDDS in comparison to Crem-SMEDDS was shown in Figure 4. Crem-SMEDDS was found to dissolute 85% of TAC within 15 min whereas

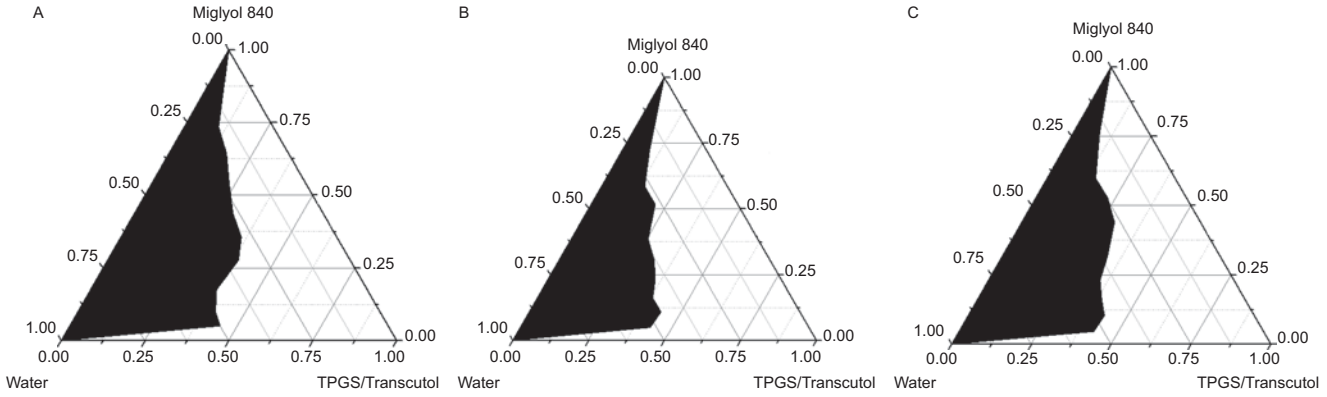

Figure 1. Pseudo-ternary phase diagram of TPGS-SMEDDS (0.8% TAC of the vehicle, w/w). (A) Miglyol840-TPGS/Transcutol (4:1); (B) Miglyol840-TPGS/Transcutol (2:1); (C) Miglyol840-PGS/Transcutol (2:1)). The blank region represents microemulsion formation area. SMEDDS, self-microemulsifying drug delivery system; TAC, tacrolimus; TPGS, tocopheryl polyethylene glycol succinate.

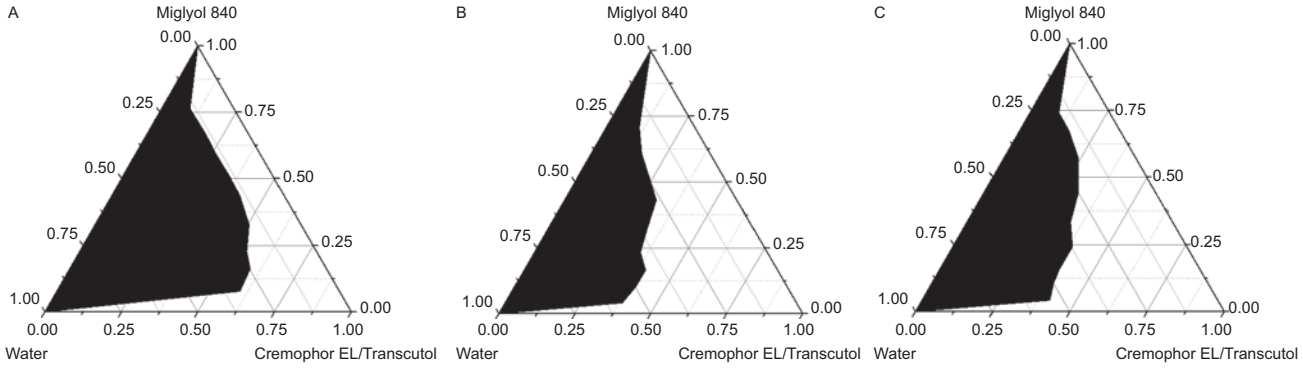

Figure 2. Pseudo-ternary phase diagram of Crem-SMEDDS (0.8% TAC of the vehicle, w/w). (A) Miglyol840-CremophorEL40/Transcutol (4:1); (B) Miglyol840-CremophorEL40/Transcutol (2:1); (C) Miglyol840-Cremophor EL40/Transcutol (2:1)). The blank region represents microemulsion formation area. SMEDDS, self-microemulsifying drug delivery system; TPGS, tocopheryl polyethylene glycol succinate.

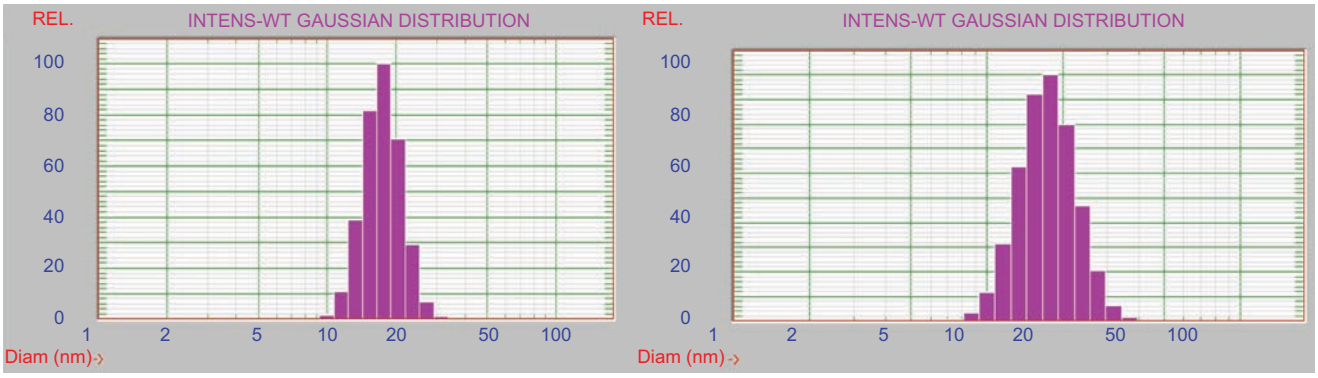

Figure 3. Size distribution of (A) TPGS-SMEDDS and (B) Crem-SMEDDS determined by a Nicomp™ 380 ZLS Zeta Potential/Particle sizer. SMEDDS, self-microemulsifying drug delivery system; TPGS, tocopheryl polyethylene glycol succinate.

TPGS-SMEDDS dissoluted less than 85% of TAC within 90 min. The dissolute rate of TAC from Crem-SMEDDS was significantly faster than that of TPGS-SMEDDS.

Bioavailability study

Figure 5 showed the blood concentration–time curves in rats after a single oral dose of TPGS-SMEDDS and Crem-SMEDDS in comparing with homemade solution. At all the indicated time points, the TAC blood concentrations in rats treated with TPGS-SMEDDS or Crem-SMEDDS were significantly higher than those treated with homemade solution. The curve of Crem-SMEDDS was slightly higher than TPGS-SMEDDS. The pharmacokinetic parameters were given in Table 2. The C_{max} and $AUC_{0\rightarrow t}$ of the SMEDDS were significantly higher than those of the homemade solution. The relative bioavailabilities of TPGS-SMEDDS and Crem-SMEDDS were approximately 7.35-fold and eightfold compared with the homemade solution. It was rational to deduce that alternative mechanisms other than improved release may contribute to enhancement of bioavailability of TAC. Moreover, the bioavailability of TPGS-SMEDDS and Crem-SMEDDS were almost alike, although the dissolution rates of them were different.

The fine microemulsion might not only increase the water solubility of drug, but also enhance accumulation in Peyer's patch for lymphatic transport of the drug, thus avoid being metabolized by the liver. The formed oil droplets also stimulated lipoprotein/chylomicron production, an effect similar to food that could apparently enhance the bioavailability of TAC[20,21]. Additionally, high content of surfactants in SMEDDS could increase the permeability by disturbing the cell membrane and demonstrated a reversible effect on the opening of tight junction[22].

Yang et al. has reported SMEDDS may decreased/inhibited P-gp drug efflux[23]. Verma et al. has demonstrated that TPGS could increase apical-to-basolateral (A-B) permeability and decrease basolateral-to-apical (B-A) permeability of paclitaxel, a substrate for P-gp. Bioavailability was enhanced about 4.2- and 6.3-fold when paclitaxel was administrated with verapamil and TPGS, respectively[24]. Yin et al. also has reported that Cremophor EL could inhibit the P-gp efflux and cause the increase A-B permeability of docetaxel[25]. Much work[18,26–28] has indicated that Cremophor EL and TPGS may inhibit the function of P-gp by affecting membrane fluidity or change its secondary and/or tertiary structure and reduce its function by specifically binding to the hydrophobic domain of the P-gp.

Conclusions

TAC is a potent immunosuppressor agent with a variable and poor oral bioavailability owing to its P-gp efflux, marked pre-systemic metabolism by CYP3A in the enerocytes and liver first pass effect[29,30]. In the present study, two SMEDDS formulations including TPGS or Cremophor EL40 were designed and evaluated for its potential to promote dissolution profile *in vitro*, and

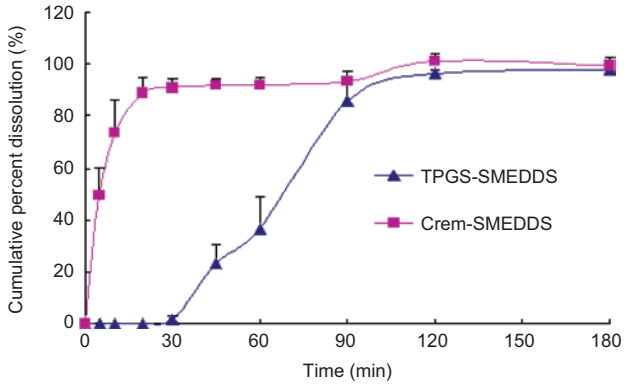

Figure 4. Dissolution profiles of TAC from TPGS-SMEDDS (closed triangles) and Crem-SMEDDS (closed squares). SMEDDS, self-microemulsifying drug delivery system; TAC, tacrolimus; TPGS, tocopheryl polyethylene glycol succinate.

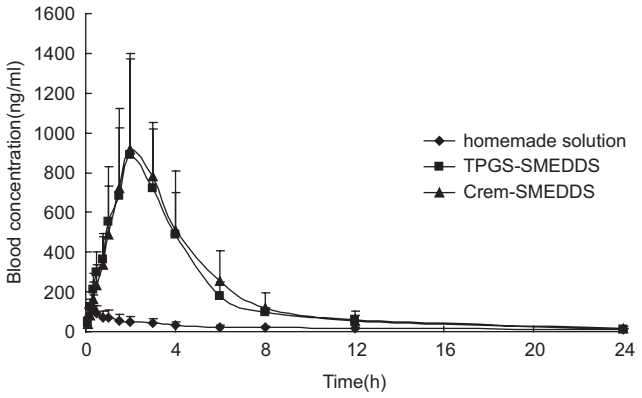

Figure 5. Mean blood concentration–time profiles of TAC after oral administration of a single dose of TPGS-SMEDDS (closed squares, 10 mg·kg^{-1}), Crem-SMEDDS (closed triangles, 10 mg·kg^{-1}) and homemade solution (open squares, 10 mg·kg^{-1}) to rats. (Each value was represented as mean ± SD, $n=6$). SMEDDS, self-microemulsifying drug delivery system; TAC, tacrolimus; TPGS, tocopheryl polyethylene glycol succinate.

Table 2. Main pharmacokinetic parameters of TAC after oral administration of TAC formulations to rats ($n=6$).

Parameters	TPGS-SMEDDS	Crem-SMEDDS	Solution
T_{max} (h)	2.5 ± 0.55*	2.5 ± 0.55*	0.29 ± 0.18
C_{max}(ng·mL^{-1})	958.36 ± 470.88*	1019.173 ± 389.62*	121.75 ± 74.37
$AUC_{0\rightarrow 24}$(ng·mL^{-1}·h)	4098.37 ± 986.96*	4469.74 ± 1664.73*	557.33 ± 235.78
Relative bioavailability(%)	735.3	802.0	—

*$p<0.05$ compared to the homemade solution group.
AUC, area under the plasma concentration–time curve; C_{max}, peak plasma concentration; SMEDDS, self-microemulsifying drug delivery system; T_{max}, time to reach the peak plasma concentration; TAC, tacrolimus; TPGS, tocopheryl polyethylene glycol succinate.

improve oral bioavailability *in vivo*. *In vitro* study indicated that dissolution rate of TAC from Crem-SMEDDS was significantly faster than that of TPGS-SMEDDS. *In vivo* bioavailability study revealed that both Crem-SMEDDS and TPGS-SMEDDS showed significant greater extent of oral absorption than the homemade solution. The relative bioavailability of TPGS-SMEDDS and Crem-SMEDDS was 735.3% and 802.0%, respectively. Although there is no clear direct evidence that the microemulsions overcame pre-systemic drug metabolism and P-gp efflux, for TAC, a significant improvement in bioavailability via oral administration might be achieved with SMEDDS.

Declaration of interest

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