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RESEARCH ARTICLE

The influence of co-solvents on the stability and bioavailability of rapamycin formulated in self-microemulsifying drug delivery systems

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Abstract

Objective: This work aims to investigate the influence of various types and different contents of co-solvent on the stability and bioavailability of rapamycin formulated in self-microemulsifying drug delivery systems (SMEDDS). *Methods*: A series of SMEDDS of rapamycin were prepared with different co-solvents [including PEG 400/ethanol (F1), glycerol/ethanol (F2), propylene glycol (F3), glycerol formal (F4), transcutol P (F5)]. Drug stability in aqueous media at different pH values and *in vitro* dispersion of SMEDDS were investigated prior to bioavailability assessment. The storage stability of rapamycin in formulations was also evaluated. *Results and discussion*: The AUC values of rapamycin following oral administration of F1, F3–F5 to rats were significantly higher than those of Rapamune® and F0 (SMEDDS without co-solvent). Interestingly, a tendency toward increased bioavailability was seen in F1–F5, which presented the better drug stability in pH 1.2 aqueous media. However, a further increase of the content of co-solvent did not effectively improve the oral bioavailability of rapamycin. Compared with F0, F1–F5 presented significant improvement of drug storage stability. More specifically, the more–OH per unit mass co-solvent had, the better stability rapamycin presented in formulation. *Conclusions*: The data obtained in present study highlight the importance of co-solvents on the stability and bioavailability of rapamycin formulated in SMEDDS. Besides solubilizing drug and increasing the dispersion rate, co-solvent could markedly affect the stability of rapamycin whether in different aqueous media or during storage and contribute to the improved oral bioavailability; it can also appropriately decrease the content of surfactant without compromising the absorption of drug.

Keywords: Self-microemulsifying drug delivery system, rapamycin, bioavailability, oral absorption, poorly water-soluble drug, stability

Introduction

Rapamycin (sirolimus, RAPA) is a carbocyclic lactone–lactam macrolide antibiotic, which has a molecular weight of 913.6 Da[1]. The drug displays a unique immunosuppressive mechanism of action through the binding with FKBP12 and the inhibition of mammalian target of rapamycin (mTOR), which induce cell-cycle arrest in the G1 phase[2,3]. These pharmacological properties allow rapamycin not only to be a promising immunosuppressant with the absence of nephrotoxicity but also to be a possible chemotherapeutic agent against many types of solid tumor[4–8].

Even though rapamycin offers promising pharmacological activities, it has low oral bioavailability (<15%) in current formulations, such as oral solution and tablet[9,10]. The low bioavailability was reported to be attributed to its sensitivity to gastric acid, partial intestinal absorption, and first-pass hepatic metabolism[11]. Rapamycin has poor water solubility (2.6 μg/mL) and high liposolubility (log $P_{O/W}$ = 5.77), and is the substrate of CYP450 3A isozymes

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and permeability glycoprotein (P-gp) existing in the small intestinal enterocytes[12,13]. These factors have been considered as significant contributors to low intestinal absorption[14,15].

In recent years, self-microemulsifying drug delivery systems (SMEDDS) have been increasingly employed to enhance the oral bioavailability of poorly water-soluble drugs and there are a number of examples of increased drug bioavailability following oral administration of SMEDDS[16–23]. Without exception, water-soluble co-solvents are included in these SMEDDS formulations and it has been assumed that co-solvents could increase the solvent capacity of the formulation for drugs that dissolve freely in co-solvents. However, in most of studies in literature, the effects of oils and surfactants on the SMEDDS performances *in vitro* and *in vivo* were mainly discussed and few studies mentioned the effect of co-solvent. Co-solvent in the SMEDDS formulation not only plays a role of drug solubilization, but also may influence the stability of drug in formulation, the dispersion profiles, and *in vivo* performances of SMEDDS.

The current study endeavors to investigate the influence of co-solvents on the stability of rapamycin when SMEDDS formulations disperse in pH 1.2 HCl solution or pH 6.8 phosphate buffer and the storage stability of drug formulated in SMEDDS. The *in vivo* performances of SMEDDS with various types and different contents of co-solvent were also evaluated.

Materials and methods

Materials

Rapamycin was purchased from Fujian Kerui Pharmaceutical Co., Ltd. (Fuzhou, China). Rapamune® (sirolimus) Oral Solution (1 mg/mL) (Wyeth Laboratories, Philadelphia, PA) was commercially available. Polyoxyl 40 hydrogenated castor oil (Cremophor RH40®) was donated by BASF, Germany. 2-(2-Ethoxyethoxy) ethanol (Transcutol P) was provided by Gattefosse, France. Glycerol, ethanol, PEG 400, and propylene glycol were obtained from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Glycerol formal was supplied from Sigma-Aldrich (St. Louis, MO). Medium-chain triglyceride (MCT) was obtained from Sins-swed Pharmaceutical Co., Ltd. (Beijing, China). All other chemicals were of analytical reagent grade except that methanol is of chromatographic reagent grade.

Preparation of rapamycin formulations

A series of SMEDDS of rapamycin were prepared with the formulations (Table 1). Rapamycin was initially dissolved by various co-solvents in glass vial with magnetic stirring at ambient temperature followed by adding Cremophor RH40 and MCT. SMEDDS without co-solvent was prepared by directly mixing drug with surfactant and oil. Then, the mixture was further stirred to ensure homogeneity. Formulations were centrifuged at 3000 *g* for 15 min and examined for signs of turbidity or phase separation prior to evaluation.

In vitro evaluation

Determination of droplet size

Each unit dose of SMEDDS containing 0.5 mg rapamycin was added in aqueous media. The effects of different dispersion media (including double-distilled water, pH 1.2 and pH 3 HCl solutions, pH 6.8 phosphate buffer) and dilution ratio (1:10, 1:50, 1:100; v/v) on the resulted microemulsion size of SMEDDS were investigated. The mean droplet size and polydispersity index (PDI) of the resultant microemulsions were determined with Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK) at 25°C.

Stability of rapamycin and rapamycin formulated in SMEDDS in different aqueous media

Rapamycin was dissolved in 1 mL of methanol and, respectively, diluted to 100 mL with HCl solutions (pH 1.2, pH 2.0, pH 3.0) and phosphate buffer (pH 6.8) preheated to 37°C. With magnetic stirring, the specific amount of media was withdrawn at scheduled time points. Samples of pH 1.2 were withdrawn every 4 min and neutralized using 0.1 M NaOH. Samples of pH 2.0 were withdrawn every half an hour and samples of pH 3.0 and pH 6.8 were collected every 12 h. Twenty microliters of sample was injected for HPLC analysis.

SMEDDS and Rapamune® were directly diluted with HCl solution (pH 1.2) and phosphate buffer (pH 6.8), respectively. The samples were stored in glass bottles maintained at 37°C. With magnetic stirring, a specific amount of media was withdrawn at scheduled time

Table 1. Composition of self-microemulsifying drug delivery system (SMEDDS) formulations.

Component	F0	F1	F2	F3	F4	F5	F6	F7
Rapamycin (mg/g)	1.58	4	4	4	4	4	4	4
MCT (g/g)	0.33	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Cremophor RH40 (g/g)	0.67	0.50	0.50	0.50	0.50	0.50	0.40	0.60
Ethanol (g/g)		0.08	0.08	—	—	—	—	—
PEG 400 (g/g)		0.17	—	—	—	—	—	—
Glycerol (g/g)		—	0.17	—	—	—	—	—
Propylene glycol (g/g)		—	—	0.25	—	—	—	—
Glycerol formal (g/g)		—	—	—	0.25	—	0.35	0.15
Transcutol P (g/g)		—	—	—	—	0.25	—	—

points. Before HPLC analysis, rapamycin was extracted with methanol from the samples.

In vitro dispersion tests

Rapamycin is relatively stable in pH 3 aqueous solution, whereas it is very sensitive to pH 1.2 solution (data presented in the section "Stability of rapamycin and rapamycin formulated in SMEDDS in different aqueous media" under Results and discussion). Thus, pH 3 HCl solution was selected as dispersion media to describe the dispersion profiles of SMEDDS. Dispersion experiments were performed in 200 mL 0.001 M HCl (pH 3.0) at 37°C using a Chinese Pharmacopoeia XC paddle method. A mass of formulation (0.25 g) was weighed onto a glass slide and introduced into the dispersion media and the paddle revolution speed was set at 50 rpm. Aliquots (0.5 mL) of the dispersion media was pipetted out at definite intervals and filtered through a membrane filter (0.22 μm). Four hundred microliters methanol was added to an aliquot sample (200 μL) and vortexed for 1 min. After centrifuging at 12,000 rpm for 1 min, 20 μL of the supernatant was injected for HPLC analysis. The mean droplet size and PDI of the resultant microemulsions presented in the media were determined with Zetasizer Nano ZS90 (Malvern Instruments Ltd.) at 25°C.

Determination of rapamycin in *in vitro* evaluation

A HPLC/UV method as described previously was employed to determine rapamycin in *in vitro* tests[24]. The HPLC analysis was performed by Dionex UVD 170U (Dionex, Chelmsford, MA), equipped with P680 HPLC pump system. The separation was achieved on a Eurospher-100 C_{18} column (250 mm × 4.6 mm, 5 μm) at a flow rate of 1.0 mL/min. The mobile phase was methanol:water (80:20). Twenty microliters of sample was injected for analysis and the effluent was monitored at 277 nm. The column temperature was set to 57°C.

Bioavailability studies

Administration and sampling

The experimental procedures were approved by the institutional animal ethical committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Sprague-Dawley rats (male, 210–250 g) were fasted for 12 h and were allocated to nine groups (five animals each) at random before the experiments. Eight groups of rats received different rapamycin SMEDDS, respectively, with a dose of 5 mg/kg by gavage. Following administration, 3 mL of water was given to animals. Rapamune® was emulsified with water before dosing, then the emulsion was orally administrated to rats with a dose of 5 mg/kg. The 0.4 mL of blood was collected from tail vein with a heparinized tube at 0, 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 h. The blood samples were stored at –20°C before analysis.

Whole blood sample preparation

The 0.4 mL whole blood was added into a 10 mL screw capped tube followed by adding 20 μL of internal standard (midazolam) solution (100 ng/mL) and 0.4 mL of 0.1 M sodium acetate pH 4.7(adjusted with acetic acid). After vortexing for 60 sec, 5 mL methyl *tert*-butyl ether was added and vortexed for 10 min. After centrifuging at 3000 rpm for 5 min, the organic layer was transferred, evaporated, and then reconstituted in 200 μL of mobile phase (methanol:water = 95:5, v:v). Then, the re-dissolved solution was centrifuged at 12,000 rpm for 5 min and 10 μL of supernatant was injected for HPLC/MS/MS analysis.

Rapamycin whole blood sample determination

Rapamycin in whole blood were quantified by liquid chromatography/tandem mass spectrometry (LC-MS/MS) using an Prominence ultraperformance liquid chromatography (UPLC) system (SHIMADZU, Japan) coupled to a 3200 Q TRAP® quadrupole linear ion trap hybrid mass spectrometer (Applied Biosystems, MDS Sciex, Foster City, CA). All chromatographic separations were performed with a Phenomenex Luna C18 column (250 mm × 2.0 mm, 5 μm). The mobile phase consisted of 95% methanol and 5% water at a total flow rate of 0.25 mL/min. The column temperature was 55°C. Electrospray ionization (ESI) source was applied and operated in the positive ion mode. Multiple reaction monitoring (MRM) mode with the transitions of m/z 936.5→409.1 and m/z 326.4→291.2 were used to quantify rapamycin and the internal standard (midazolam), respectively.

Pharmacokinetic data analysis

The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual whole blood concentration versus time profiles. The area under the whole blood concentration–time curve ($AUC_{0\to t}$) was estimated by linear trapezoidal method.

Storage stability of rapamycin formulated in SMEDDS

The storage stability of rapamycin SMEDDS formulations were investigated at 4°C, 25°C, and 40°C, respectively. Before sampling, formulations were examined for signs of turbidity or phase separation. The samples of 40°C and 25°C were withdrawn at 1, 2, 3, 5, 10, 20, 40, 60, 90 days. The samples of 4°C were withdrawn at 1, 5, 10, 20, 40, 60, 90 days. The samples diluted with methanol before HPLC analysis. In addition, the droplet sizes of the microemulsions resulted from the samples of 4°C and 25°C dispersing in double-distilled water were determined at 0, 1, 10, 40, 90 days, respectively.

Statistical analysis

Results are reported as mean ± standard deviation (SD). Statistically significant differences were determined by ANOVA followed by Tukey's test for multiple comparisons at a significance level of $P = 0.05$.

Results and discussion

Formulation and *in vitro* dispersion of SMEDDS

In our previous study, MCT presented better solubility of rapamycin [≈2.3 mg/mL, about 4-fold higher than long-chain triglyceride (LCT)] and tended to be emulsified easily[24]. Compared with Cremophor EL and Tween 80, Cremophor RH40 showed a larger self-microemulsifying region in pseudo-ternary phase diagrams[24]. Therefore, MCT and Cremophor RH40 were selected as the oil and surfactant to prepare the SMEDDS formulations. To increase rapamycin solubility in formulations, various co-solvents were used to solubilize rapamycin. Although co-solvents exhibited different solubilizing ability, they all were found to be able to solubilize rapamycin to an amount of prescription. But, in contrast, formulation without co-solvent (F0) was found to solubilize only ≈40% of the amount of prescription (Table 1). In order to achieve superior microemulsions with similar droplets size and efficient dispersion of MCT formulations, the optimal formulations with 50% (w/w) Cremophor RH40 and 25% (w/w) co-solvent were selected (Table 1). Transcutol P and glycerol formal showed an excellent compatibility with MCT and Cremophor RH40 due to its lower viscosity and better solvent properties. As for propylene glycol, although it was less compatible with oil and surfactant, an isotropic mixture was formed with a longer time of agitation. Glycerol and PEG 400 were not compatible with other formulation components to form a single phase system and the problem can be solved by adding ≈8% w/w ethanol to these incompatible formulations.

When SMEDDS formulations dispersed in various aqueous media (including double-distilled water, pH 1.2, pH 2.0, and pH 3.0 HCl solutions, pH 6.8 phosphate buffer) according to different dilution ratios, no significant differences were observed in the mean droplet sizes of the resultant microemulsions (data not shown). The results indicated that the mean droplet sizes of microemulsions resulted from SMEDDS were not affected by pH, the ionic strength, and the volume of dispersion medium, which is consistent with previous findings[23,24].

All the SMEDDS formulations except F0 dispersed rapidly (>90% within 30 min) (Figure 1) to form microemulsions with emulsion droplets size <50 nm and maintained rapamycin in a solubilized state at the end of the 60-min dispersion.

The dispersion profiles of SMEDDS formulations fit the Weibull model and T_{50} and T_d were calculated (Table 2). Statistically significant differences were observed between F0 and other SMEDDS formulations ($P < 0.05$), and the T_{50} and T_d values of F2 were also found to be significantly different from other SMEDDS formulations. The dispersion rate of SMEDDS formulation without co-solvent was found to be slower than other formulations, which indicated that co-solvents not only play a role of drug solubilization but also promote the dispersion rate of SMEDDS formulations. In the present study, co-solvents used are all hydrophilic substances, which can mix with water at any proportion. Therefore, when SMEDDS formulations disperse in the aqueous media, co-solvent can accelerate the dispersion of surfactant and further improve the emulsification speed. However, in the range of 15% to 35%, an improvement in content of co-solvent did not lead to an enhancement of dispersion rate (F4, F7 vs. F6), which indicated that more content of co-solvent in formulation cannot help further improve the dispersion of formulation.

Stability of rapamycin and rapamycin formulated in SMEDDS in different aqueous media

Rapamycin has been shown to be sensitive to both acid and base, resulting in ring fragmentation and degradation[25–27]. Although ring-opened compounds retain high affinity to FKBP12, all the derivatives lacking macrocycle exhibited extremely weak immunosuppressive activity[28,29]. Therefore, better stability of rapamycin in simulated gastrointestinal pH environment will contribute to better drug absorption and thereby to higher immunosuppressive activity.

Degradation kinetics of rapamycin and rapamycin formulated in SMEDDS in aqueous solutions with different pH values fit the first-order equations. The values of half-lives were shown in Table 3. Rapamycin was extremely unstable in pH 1.2 HCl solution ($t_{1/2\,(\mathrm{pH}\,1.2)} \approx 10$ min) and the stability was improved significantly with the increasing pH value of HCl solution ($t_{1/2\,(\mathrm{pH}\,2)} \approx 36$ min, $t_{1/2\,(\mathrm{pH}\,3)} \approx 672$ min, $P < 0.05$). Furthermore, rapamycin was found to be unstable in phosphate buffer (pH 6.8) at 37°C, which was consistent with the results reported earlier[30]. However, the stability of rapamycin presented >4-fold enhancement (data not shown) in the phosphate buffer solution diluted 10-fold with double-distilled water (pH 6.8). The result clearly shows that the degradation of rapamycin at neutral pH was strongly accelerated at higher buffer concentration. Same results were observed by Il'ichev et al.[31]. Both specific and general base catalysis were proposed to be the mechanisms of rapamycin degradation in aqueous solution[31]. Therefore, ions have an important influence on the stability of rapamycin. When rapamycin is studied in aqueous media, buffer concentration should be kept as low as possible to minimize degradation.

Importantly, the resulting microemulsions from SMEDDS increased the drug stability significantly in both pH 1.2 HCl solution and pH 6.8 phosphate buffer ($P < 0.05$). Compared with the stability of free drug, the drug stability presented an approximately 3.2- to 7.6-fold improvement in pH 1.2 aqueous media and about 6.5- to 10-fold enhancement in pH 6.8 phosphate buffer. Therefore, it is rational to deduce that the resulting microemulsions from SMEDDS could partly protect rapamycin from degradation in gastrointestinal tract, which may contribute to the drug absorption. Interestingly, although Rapamune® showed a better drug-protective effect than SMEDDS in pH 1.2 solution, there was no apparent improvement in drug stability in pH 6.8 phosphate buffer

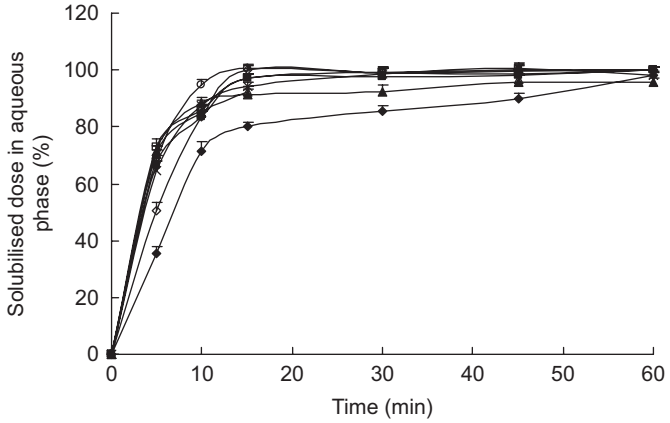

Figure 1. Dispersion profiles of rapamycin SMEDDS [F0 (-◆-), F1 (-■-), F2 (-▲-), F3 (-×-), F4 (-◊-), F5 (-□-), F6 (-Δ-), and F7 (-*-)] in pH 3 HCl solution (mean ± SD, $n=3$).

Table 2. Dispersion parameters and particle size obtained in dispersion tests (mean ± SD, $n=3$).

	T_{50}	T_d	Particle size (nm)	Polydispersity
F0	6.32 ± 0.89*	10.71 ± 1.52*	35.38	0.101
F1	1.81 ± 0.26	3.36 ± 0.49	42.66	0.151
F2	0.57 ± 0.08**	1.65 ± 0.20**	37.22	0.099
F3	1.65 ± 0.22	3.18 ± 0.45	42.93	0.208
F4	2.81 ± 0.39	4.40 ± 0.63	33.18	0.089
F5	2.12 ± 0.25	3.48 ± 0.43	40.91	0.106
F6	2.46 ± 0.28	4.44 ± 0.62	28.56	0.073
F7	1.75 ± 0.26	3.04 ± 0.43	58.24	0.170

*$P<0.05$, compared with SMEDDS formulations including various co-solvents.
**$P<0.05$, compared with SMEDDS formulations including other co-solvents.

Table 3. Half-lives of rapamycin, rapamycin formulated in self-microemulsifying drug delivery systems (SMEDDS), and Rapamune® in different aqueous media (mean ± SD, $n=3$).

Formulations	pH 1.2 HCl solution (h)	pH 6.8 phosphate buffer (h)
Rapamycin	0.178 ± 0.019	3.77 ± 0.19
F0	1.156 ± 0.069	35.88 ± 1.79
F1	1.360 ± 0.082	34.70 ± 1.72
F2	0.586 ± 0.037	30.13 ± 1.52
F3	0.689 ± 0.041	33.43 ± 1.48
F4	1.022 ± 0.066	37.81 ± 1.89
F5	1.288 ± 0.063	36.21 ± 1.46
F6	1.105 ± 0.072	37.23 ± 1.63
F7	0.982 ± 0.054	36.65 ± 1.70
Rapamune®	2.713 ± 0.256	4.70 ± 0.36

when compared Rapamune® with free drug, suggesting that the ingredients in Rapamune® were more sensitive to pH 6.8 phosphate buffer than that in SMEDDS.

Bioavailability studies

The *in vivo* studies were performed to investigate pharmacokinetic behavior of rapamycin following oral administration of Rapamune® commercially available and SMEDDS forms. The tail incision method was used to collect blood from rats. This method was considered to be a validated animal-friendly alternative to other conventionally used methods and techniques for collecting blood from laboratory rats[32]. Figures 2 and 3 illustrate the whole blood concentration versus time profiles of rapamycin following the administration of nine forms. The pharmacokinetic parameters are presented in Table 4.

The C_{max} values of rapamycin following oral administration of all SMEDDS formulations whether it contained the co-solvents or not were significantly higher than those of rapamycin oral solution (Rapamune®) ($P<0.05$). Besides, the C_{max} value of rapamycin was markedly improved ($P<0.05$) when co-solvents were included in the SMEDDS formulations except F2 (F1, F3–F7 vs. F0). Similarly, the AUC values of rapamycin following oral administration of the SMEDDS formulations including co-solvents except F2 were significantly higher than those of rapamycin oral solution (Rapamune®) and the SMEDDS formulation without co-solvent (F0) ($P<0.05$). Although there were no statistically significant differences among the AUC values of F0, Rapamune® and F2 ($P>0.05$), the C_{max} and AUC values of F2 tended to be slightly higher than Rapamune® and F0. These special results might be explained by the significantly rapid dispersion of F2 (Table 2) and the limited protection effect of resultant microemulsion on drug (Table 3). Moreover, no significant differences in C_{max} and AUC values were observed among the SMEDDS formulations including the same content of various co-solvents (F1–F5, $P>0.05$), which indicated that under the precondition of effective dispersion, co-solvents have no significant influence on the *in vivo* performance of SMEDDS at current ratios.

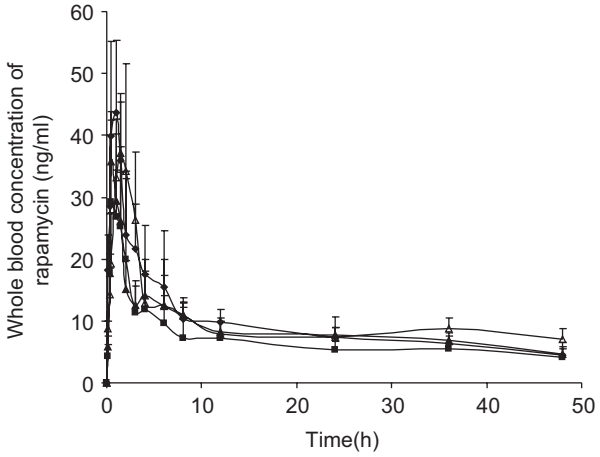

Figure 2. Whole blood concentration versus time profiles of rapamycin after oral administration of self-microemulsifying drug delivery system (SMEDDS) formulations [F1 (-◆-), F2 (-■-), F3 (-▲-), and F5 (-Δ-)] to rats ($n = 5$ and 5 mg/kg).

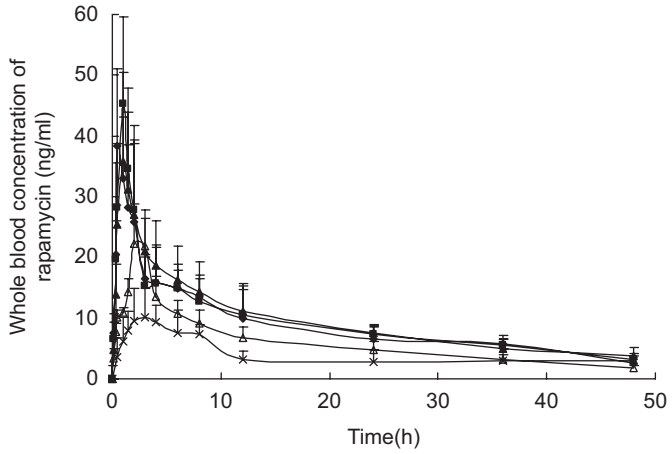

Figure 3. Whole blood concentration versus time profiles of rapamycin after oral administration of self-microemulsifying drug delivery system (SMEDDS) formulations [F0 (-Δ-), F4 (-■-), F6 (-◆-), F7 (-▲-), and Rapamune® (-×-) to rats ($n = 5$ and 5 mg/kg)].

Table 4. Relative bioavailability and pharmacokinetic parameters obtained following oral administration of rapamycin in different formulations to rats ($n = 5$, mean ± SD).

		Self-microemulsifying drug delivery systems (SMEDDS)							
	Rapamune®	F0	F1	F2	F3	F4	F5	F6	F7
$AUC_{0\to48\,h}$ (ng·h/mL)	190.28 ± 85.99	283.96 ± 60.58	459.00 ± 68.77*	335.67 ± 97.82	410.42 ± 49.94*	440.95 ± 57.47*	478.47 ± 122.06*	420.19 ± 54.72*	451.44 ± 62.89*
C_{max} (ng/mL)	12.04 ± 4.30	25.88 ± 3.54**	48.42 ± 10.87**,***	33.92 ± 12.14**	38.57 ± 3.56**,***	46.06 ± 13.26**,***	43.59 ± 6.85**,***	42.85 ± 10.64**,***	38.29 ± 9.85**,***
T_{max} (h)	2.60 ± 0.55	2.60 ± 0.55	0.90 ± 0.42*	1.00 ± 0.50*	1.00 ± 0.50*	0.80 ± 0.27*	1.60 ± 0.42*	0.80 ± 0.27*	1.00 ± 0.50*
Relative bioavailability (%)	—	1.49 ± 0.32	2.41 ± 0.36	1.76 ± 0.51	2.18 ± 0.22	2.32 ± 0.20	2.51 ± 0.64	2.21 ± 0.25	2.37 ± 0.32

*$P < 0.05$, compared with Rapamune® and the SMEDDS formulation without co-solvent (F0).
**$P < 0.05$, compared with Rapamune®.
***$P < 0.05$, compared with the SMEDDS formulation without co-solvent (F0).

Interestingly, a tendency toward increased bioavailability was seen in the SMEDDS formulations including the same content of various co-solvents (F1–F5), which presented the better drug stability in pH 1.2 aqueous media, although the difference of AUC values were not significant. Rapamune® presented the better drug stability in pH 1.2 aqueous media compared with all SMEDDS formulations; however, its stability in pH 6.8 phosphate buffer was shown to be still poor, which contribute to the lower oral bioavailability of rapamycin. These results confirm the viewpoint that better stability of rapamycin in simulated gastrointestinal pH environment will contribute to better drug absorption.

Compared with F0 and Rapamune®, the T_{max} values of F1–F7 were significantly decreased ($P < 0.05$), which means that co-solvent can effectively increase the absorption rate of drug.

In addition, when the content of co-solvent increased from 15% to 35% and the content of surfactant decreased from 60% to 40%, no significant differences in the C_{max}, T_{max}, and AUC values were observed even though the particle size of the resultant microemulsion became larger (F4 vs. F6 vs. F7; Table 2). The results indicated that an increase of the content of co-solvent did not effectively improve the oral bioavailability of rapamycin within a certain range. But from another point of view, an appropriate decrease of the content of surfactant in the SMEDDS formulation, on the one hand, did not compromise the absorption of drug and on the other hand may reduce the risk of gastrointestinal irritation induced by the large quantity of surfactants in the SMEDDS formulations.

The enhancement of bioavailability of rapamycin might be attributed to several aspects. First, the high content of surfactants used in formulations of SMEDDS could improve the intestinal drug solubilization and intestinal drug permeability, which determined the extent of oral absorption and the oral bioavailability in basic terms[33,34]. Second, the mean particle size of emulsions from SMEDDS (mean droplet size: <50 nm) was much smaller than that from oral solution (mean droplet size: ≈500 nm). Microemulsion generates a high surface area of interaction between the droplets and gastrointestinal tract fluids, offering an improvement in the rate and extent of absorption and resulting in better oral bioavailability[35]. Third, SMEDDS showed a better drug-protective effect than Rapamune® in simulated intestinal pH environment and the small intestine was undoubtedly the main site of drug absorption. Last but not least, the inhibitory effect of excipients (such as Cremophor RH40) on cytochrome P450 (CYP) 3A isozymes and/or P-gp functionality has been suggested to explain the increase in bioavailability of several poorly water-soluble substrates of CYP3A or P-gp, such as talinolol and saquinavir[36–40]. In the same way, SMEDDS with Cremophor RH40 may enhance the oral absorption of rapamycin, the substrates of CYP 3A and P-gp, by inhibiting the CYP3A metabolism and P-gp transport[41,42]. Therefore, SMEDDS could increase bioavailability of rapamycin compared with oral solution and might be a promising approach to the oral delivery of rapamycin.

In summary, the SMEDDS formulations afforded a 1.5- to 2.5-fold improvement in oral bioavailability relative to oral solution. Co-solvent can effectively improve the absorption rate and extent of drug from SMEDDS formulations although a further improvement of content of co-solvent did not further enhance the oral bioavailability of rapamycin.

Storage stability of rapamycin formulated in SMEDDS

Within 90 days, all SMEDDS formulations did not present any signs of turbidity or phase separation and the droplets size of the resultant microemulsions from the samples remained <50 nm (RSD < 5%) even though rapamycin presented different chemical stability in various SMEDDS formulations at different storage temperatures. Degradation kinetics of rapamycin formulated in SMEDDS at different storage temperatures were also complied with the first-order equations. The values of half-lives are shown in Table 5. The storage stability was improved significantly ($P < 0.05$) with decreasing temperature suggesting that SMEDDS formulations of rapamycin should be stored at lower temperature. Compared with F0, SMEDDS formulations with various co-solvents presented significant improvement of drug stability. Moreover, glycerol and propylene glycol showed a better effect of drug protection compared with other co-solvents even though their solubilization effect was less than transcutol P and glycerol formal.

More interestingly, the more–OH per unit mass co-solvent had, the better stability rapamycin presented in formulation (Table 5). There was a good correlation between the half-lives of drug and the–OH content that co-solvent has in unit mass SMEDDS formulation (25°C, $r = 0.9555$; 40°C, $r = 0.9652$; F1–F5; Table 5). Furthermore, the more content of co-solvent in SMEDDS, the better stability drug presented (F7 vs. F4 vs. F6; Table 5). The use and quantitative analysis of rapamycin pose many challenges associated with facile degradation and the multitude of isomeric forms[31]. Based on the results of X-ray crystallographic

Table 5. Half-lives of rapamycin formulated in self-microemulsifying drug delivery systems (SMEDDS) at different storage temperatures (mean ± SD, $n = 3$).

Formulations	–OH content (mmol/g)[a]	4°C (days)	25°C (days)	40°C (days)
F0	—	10.43 ± 0.58	0.67 ± 0.03	0.19 ± 0.01
F1	—	426.71 ± 38.90	10.13 ± 0.82	2.85 ± 0.14
F2	7.28	—[b]	116.20 ± 3.00	23.57 ± 1.34
F3	6.57	—[b]	92.84 ± 1.91	16.40 ± 0.59
F4	2.40	—[b]	47.71 ± 3.13	7.12 ± 0.32
F5	1.86	80.91 ± 1.45	5.06 ± 0.37	1.10 ± 0.07
F6	3.36	—[b]	61.16 ± 3.57	10.08 ± 0.65
F7	1.44	952.65 ± 52.40	26.13 ± 1.22	4.75 ± 0.21

[a]The–OH content refers to the–OH content that co-solvent has in unit mass SMEDDS.
[b]The content of rapamycin in F2, F3, and F4 remained unchanged in 90 days (RSD% < 5%).

studies of the purified compound and its complexes with FKBP-12, a representative structural formula was named as *trans*-rotamer of 10-hemiketal[43,44]. Ring cleavage is a typical reaction that rapamycin affords under a variety of experimental conditions via β-elimination to form enone or via hydrolysis of the lactone, which leads to significant loss of antiproliferative and immunosuppressive activity, despite the fact that binding to FKBP-12 is only slightly reduced[28,45]. As mechanistic studies are complicated by *cis–trans* isomerization around the amide bond and by isomerization associated with the hemiketal formation, the kinetics and mechanism of formation and degradation in protic solvents are not well understood[31]. Despite the precise mechanism needs to be elucidated by further research, the results in the present study at least indicated that polyhydric alcohol may protect rapamycin from degradation.

Conclusions

The data obtained in the present study highlight the importance of co-solvents on the stability and bioavailability of rapamycin formulated in SMEDDS. The data in storage stability tests suggest that different co-solvents had a significant impact on the stability of rapamycin. Interestingly, the more–OH per unit mass co-solvent had, the better stability rapamycin presented in formulation. Compared with commercially available oral solution (Rapamune®) and co-solvent-free SMEDDS formulation (F0), SMEDDS formulations including different co-solvents (F1, F3–F7) offered a significant improvement in the rate and extent of absorption and resulted in better oral bioavailability. These results demonstrate that co-solvent can effectively improve the absorption rate and extent of drug from SMEDDS formulations and illustrate the potential use of SMEDDS for the delivery of rapamycin by the oral route. According to the results in the present study, propylene glycol and glycerol formal could be the better choices of co-solvent for rapamycin SMEDDS formulations based on their better storage stability and higher *in vivo* bioavailability of drug.

In the present study, co-solvents exercise influence on the SMEDDS formulation at least from four aspects: (1) to solubilize rapamycin to an amount of prescription; (2) to affect the stability of drug whether in aqueous media at different pH values or during storage; (3) to aid dispersion of systems which contain a high proportion of surfactant; and (4) to appropriately decrease the content of surfactant in the SMEDDS formulation without compromising the absorption of drug.

In previous studies on the SMEDDS formulations, the role of co-solvent has been underestimated. The results of this study confirmed the previous assumption that co-solvent in the SMEDDS formulation not only plays a role of drug solublization, but also may influence the stability of drug in formulation, the dispersion profiles, and *in vivo* performances of SMEDDS. Since the co-solvent plays a significant role in the SMEDDS formulation, how to select appropriate co-solvent is important with regard to the design of SMEDDS formulation. Besides the solvent capacity, miscibility with other components and role in promoting self-dispersion of the formulation, drug stability whether formulation dispersing in simulated gastrointestinal pH environment or during storage is another important design criterion for the screening of co-solvent and the development of SMEDDS formulation, especially for the drugs with poor chemical stability, such as rapamycin.

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Declaration of interest

All authors have no competing interest to declare.

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