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Solubilization of rapamycin

Pahala Simamora [a,*], Joan M. Alvarez [b], Samuel H. Yalkowsky [b]

[a] *Pharmaceutical Research and Development, Schering-Plough HealthCare Products, Inc., 3030 Jackson Avenue, Memphis, TN 38151, USA*

[b] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA*

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Abstract

The solubilization of rapamycin, a poorly water soluble investigational immunosuppressive drug, by facilitated hydrotropy is presented. Partially water-miscible aromatic solutes (such as benzyl alcohol, benzoate, or benzoic acid) can be solubilized by water-miscible cosolvents, such as ethanol and propylene glycol. Once solubilized, the partially miscible aromatic solute becomes a solubilizing agent. This technique yielded a dramatic (> 1000-fold) increase in the aqueous solubility of rapamycin. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Rapamycin is a carboxylic lactone-lactam macrolide derived from the actinomycete, *Streptomyces hygroscopicus* (Sehgal et al., 1975; Vezina et al., 1975; Singh et al., 1979). It has been shown to have potent immunosuppressive activity and to inhibit T-cell activation and proliferation (Martel et al., 1977; Bierer et al., 1990; Dumont et al., 1990; Kahan et al., 1991; Blazer et al., 1994; Sehgal, 1998). Data from preclinical studies suggest that rapamycin is a potent immunosuppressive drug that could add significantly to graft survival time (Stepkowski and Kahan, 1991; Stepkowski et al., 1991; Blazar et al., 1993). Fig. 1 illustrates the chemical structure of rapamycin. Early clinical investigation required injectable i.v. formulations for administration via direct bolus injection with the desired dosing concentration of 1.0 mg/ml, representing a solubility increase of nearly 400-fold.

Rapamycin is practically insoluble in water and contains no functional groups that are ionizable in the pH range of 1–10 (unpublished report). The solubility in water was determined to be 2.6 μg/ml. Rapamycin is only slightly soluble in acceptable parenteral excipients such as ethanol, propylene glycol, glycerine, Polysorbate 80 and polyethylene glycol 400 (Waranis and Leonard, 1997). Consequently, clinically and commercially acceptable injectable formulations of rapamycin have been difficult to make. This study addresses

* Corresponding author. Tel.: +1-901-3202332; fax: +1-901-3203105.

E-mail address: pahala.simamora@spcorp.com (P. Simamora).

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the solubilization of rapamycin by facilitated hydrotropy utilizing pharmaceutically acceptable excipients. Facilitated hydrotropy is a unique strategy for solubilization in which one or more completely-water-miscible cosolvents is used to solubilize a partially water miscible (often aromatic) solute which in turn acts to further solubilize the drug (Gupta et al., 1991; Yalkowsky, 1999).

2. Materials and methods

2.1. Materials

Rapamycin was supplied by Wyeth-Ayerst Research (Rouses Point, New York) and used as received. Double distilled water was used in all experiments. All other solvents and chemicals were of reagent or HPLC grade and were used as supplied from commercial suppliers.

2.2. Methods

2.2.1. Preliminary solubility study of rapamycin

Preliminary solubility measurements were conducted to obtain the approximate solubility of rapamycin in pure organic solvents. A known amount of rapamycin (approximately 2–3 mg) was transferred into a small vial and 0.1 ml of cosolvent was added and the solution was agitated. More rapamycin was added until either saturation was reached or about 10 mg of drug has been added to the 0.1 ml cosolvent. The cosolvents used are listed in Table 1.

Table 1
Approximate solubility of rapamycin in pure cosolvents

Cosolvent	Approximate solubility (mg/ml)
Polyethylene glycol 400 (PEG 400)	<30
Propylene glycol (PG)	<15
Glycerin	<10
Triacetin	<20
Diacetin	<20
Corn oil	<1
Acetyl triethyl citrate	<20
Ethyl lactate	<20
Polyglycolated capryl glyceride (Labrasol)	<20
Ethanol	>90
N-Methyl-2-pyrrolidinone (NMP)	>120
γ-Butyrolactone	>100
Dimethyl isosorbide (DMI)	>110
Tryethylene glycol dimethyl ether (Triglyme)	>100
Ethoxy diglycol (Transcutol)	>100
Glycerol formal	>100
Dimethyl formamide (DMF)	>300
Dimethyl acetamide (DMA)	>500
Dimethyl solfoxide (DMSO)	>250
Benzyl alcohol	>400

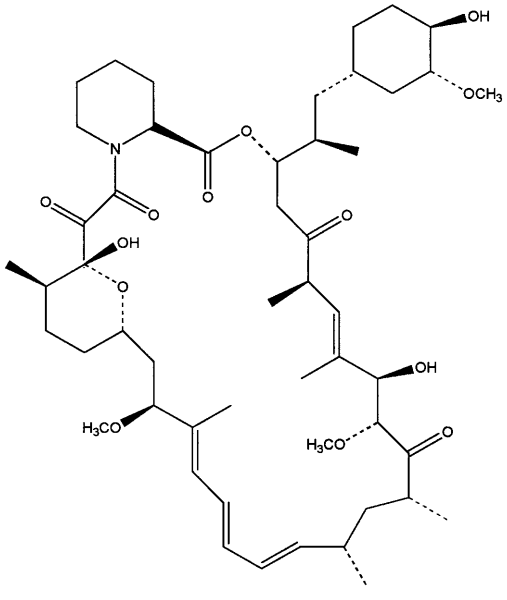

Fig. 1. Chemical structure of rapamycin.

2.2.2. Measurement of the solubility of rapamycin in cosolvent–water mixtures

An excess amount of rapamycin powder was added to vials containing solutions with different percentages of either pure cosolvent(s) or cosolvents in combination with hydrotropes. The latter were benzyl alcohol and benzoate buffer; which consists of equal amount of benzoic acid and sodium benzoate. The percentages of cosolvent and cosolvent–hydrotrope combinations are

Table 2
Solubility of rapamycin (mg/ml) in single cosolvent–water mixtures

Cosolvent	% of cosolvent (v/v)		
	20	30	40
Ethanol	0.009	–	–
DMI	0.016	0.048	0.297
NMP	0.025	0.047	0.261
γ-Butyrolactone	0.063	0.578	3.754
Glycerol formal	0.014	0.032	0.176
Transcutol	0.015	0.027	0.158
Triglyme	0.011	0.017	0.080
Polysorbate 80	0.790	–	–

given in Tables 2 and 3, respectively. The saturated solutions were equilibrated by placing them on an end-over-end mechanical rotator at 25 rev./min and at 25°C for 72 h in the absence of light. Samples with undissolved drug present were considered to have reached equilibrium and were removed from the rotator. Samples were then filtered through a 0.5-μm Millex-LCR$_{13}$ filter (Millipore) and diluted properly with the HPLC mobile phase before injection into the HPLC system.

2.2.3. HPLC assay

A Beckman Gold HPLC system equipped with a model no. 168 detector at 277 nm was used for all assays. A 250-mm Econosphere C8, 5-μm column (Alltech) equipped with 15 × 3.2 mm C8, 7-μm Brownlee guard column (ABI Analytical) were used with a mobile phase composed of 80% (v/v) of methanol in water. The flow rate was controlled at 1 ml/min. The injection volume was 100 μl. The retention time of rapamycin was approximately 7.5 min. The stock solution was prepared by dissolving rapamycin in pure methanol and stored in the refrigerator in an amber flask. The standard solutions were prepared by serial dilution of the stock solution with the mobile phase. The calibration curve was found to be linear ($r = 0.998$) in the range 1–100 μg/ml. Linearity and reproducibility were assessed by duplicate injections of six standards.

3. Results and discussions

As mentioned previously, the solubility of rapamycin in water is only 2.6 μg/ml, which is well below the target solution concentration of 1 mg/ml. Since the molecule has no ionizable functional groups, pH adjustment has no effect on its aqueous solubility. The approximate solubility of rapamycin in pure cosolvents is shown in Table 1. Several solvents were found to be able to solubilize rapamycin to a concentration of greater than 90 mg/ml. However, as shown in Table 2, upon

Table 3
Solubility of rapamycin in multiple cosolvent and hydrotrope mixtures

% Ethanol	% Propylene glycol	% Benzyl alcohol	% Benzoate buffer	Solubility (mg/ml)
0	0	0	0	0.0026
10	40	0	0	0.23
10	40	5	0	3.00
10	40	0	5	4.40
10	40	1.5	5	5.42*
10	40	3	5	6.83
10	40	5	1.5	8.42
10	40	5	3	10.65
10	40	5	5	11.26
0	0	5	0	**
0	0	0	5	**
0	0	1.5	5	**

* Vehicle used in the current valium injectable (Medical Economics, 1999).
** These vehicles cannot be prepared.

mixing with water, the aqueous solubility of the drug decreases substantially to the level below the desired concentration. Only γ-butyrolactone (at 40%) yielded rapamycin solubility greater than 1 mg/ml. Despite its solubilizing ability, γ-butyrolactone is not pharmaceutically acceptable for use in parenteral preparations.

Interestingly, benzyl alcohol yielded extremely high rapamycin solubility (>400 mg/ml). Although not normally regarded as a cosolvent, this excipient is used in numerous parenteral products as a preservative (Wang and Kowal, 1980; Strickley, 1999). Benzyl alcohol is only partially soluble. Its aqueous solubility is approximately 40 mg/ml (Merck, 1996). Because of its remarkable solubilizing power, the solubilization approach focused on the use of benzyl alcohol as the primary solubilizer. Other aromatics with similar physicochemical properties such as benzoic acid would also be expected to possess similar solubilizing power toward rapamycin. The combination of benzyl alcohol and benzoic acid would be expected to produce an additive and hopefully a synergistic effect. Benzoic acid is poorly water soluble solid with an aqueous solubility of only about 3.4 mg/ml (Merck, 1996). The low water solubility of these two aromatic hydrotropes, however, can be enhanced by the use of conventional cosolvents. Additionally, the more water soluble salt form of benzoic acid (sodium benzoate) can also be used to improve the solubility. In this study the benzoate buffer was employed, composed of equal amount of benzoic acid and sodium benzoate.

The solubility of rapamycin in multiple cosolvent and hydrotrope mixtures is shown in Table 3. The use of 10% ethanol and 40% propylene glycol yielded a drug solubility increase by two orders of magnitude. A dramatic solubility increase is obtained when small amounts of either benzyl alcohol or benzoate buffer is incorporated into an ethanol–propylene glycol solution. The drug's solubility is increased by another order of magnitude. The data from Table 3 also show that the combined incorporation of 5% of each of the relatively insoluble hydrotropes produces a nearly two orders of magnitude increase in solubility over that of the cosolvent mixture.

The results show that the incorporation of hydrotropes (either individually or in combination) into the ethanol–propylene glycol solution yields substantially greater drug solubility than that of the ethanol–propylene glycol solution alone. In fact, the drug solubility is well above the desired concentration. The use of hydrotrope combinations yields higher rapamycin solubility than that of the single hydrotrope, and the higher the hydrotrope concentration in the solution the more drug can be solubilized. Note that a 5% solution of either benzyl alcohol or benzoate buffer in water cannot be prepared due to their limited solubility.

The two conventional cosolvents were incorporated in the formulations primarily to solubilize the two aromatic hydrotropes. They are good solubilizers for the hydrotropes but not for the drug. Once the two aromatics are solubilized, they are very effective as rapamycin solubilizers.

In conclusion, conventional cosolvents such as ethanol and propylene glycol were used to solubilize partially miscible hydrotropic agents, benzyl alcohol and benzoate buffer, to provide adequate rapamycin solubility without the use of unapproved excipients.

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