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RAPAMYCIN FORMULATIONS FOR ORAL ADMINISTRATION

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(57) Claim

1. A composition of matter for oral administration containing from 0.01 gram to 10.0 grams of rapamycin per 100 ml of composition, and a solvent system, said solvent system comprising from 0.05 to 10% by volume of surfactant, from 0 to 25% by volume of inert solvent and from 65 to 99.95% by volume of phospholipid solution in which phospholipid in said solution is from 40% to 75% by weight.

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RAPAMYCIN FORMULATIONS FOR ORAL ADMINISTRATION

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The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

RAPAMYCIN FORMULATIONS FOR ORAL ADMINISTRATION

This invention relates to formulations containing rapamycin, or pharmaceutically acceptable salts of rapamycin, which are useful in oral administrations for inducing immunosuppression and for treating transplantation rejection, host vs. graft disease, autoimmune diseases, diseases of inflammation, solid tumors, fungal infections, adult T-cell leukemia/lymphomas and hyperproliferative vascular disorders. This invention also relates to processes for preparing such compositions.

Rapamycin is a macrolide antibiotic produced by *Streptomyces hygroscopicus* which was discovered first for its properties as an antifungal agent. It adversely affects the growth of fungi such as *Candida albicans* and *Microsporum gypseum*. Rapamycin, its preparation and its antibiotic activity were described in U.S. Patent No. 3,929,992, issued December 30, 1975 to Surendra Sehgal et al. In 1977 Martel, R. R. et al. reported on immunosuppressive properties of rapamycin against experimental allergic encephalitis and adjuvant arthritis in the Canadian Journal of Physiological Pharmacology, 55, 48-51 (1977). In 1989, Calne, R. Y. et al. in Lancet, 1989, no. 2, p. 227 and Morris, R. E. and Meiser, B. M. in Medicinal Science Research, 1989, No. 17, P. 609-10, separately reported on the effectiveness of rapamycin in inhibiting rejection *in vivo* in allograft transplantation. Numerous articles have followed describing the immunosuppressive and rejection inhibiting properties of rapamycin, and clinical investigations have begun for the use of rapamycin in inhibiting rejection in transplantation in man.

Rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in

preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Patent 5,100,899].

Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Patent 5,078,999], pulmonary inflammation [U.S. Patent 5,080,899], insulin dependent diabetes mellitus [Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract), (1990)], and smooth muscle cell proliferation and intimal thickening following vascular injury [Morris, R. J. Heart Lung Transplant 11 (pt. 2): 197 (1992)].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Patent 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Patent 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions. U.S. Patent 5,118,678 discloses carbamates of rapamycin that are useful as immunosuppressive, anti-inflammatory, antifungal, and antitumor agents. U. S. Patent 5,100,883 discloses fluorinated esters of rapamycin. U. S. Patent 5,118,677 discloses amide esters of rapamycin. U. S. Patent 5,130,307 discloses aminoesters of rapamycin. U. S. Patent 5,117,203 discloses sulfonates and sulfamates of rapamycin. U. S. Patent 5,194,447 discloses sulfonylcarbamates of rapamycin.

U.S. Patent No. 5,100,899 (Calne) discloses methods of inhibiting transplant rejection in mammals using rapamycin and derivatives and prodrugs thereof. Other chemotherapeutic agents listed for use with rapamycin are azathioprine, corticosteroids, cyclosporin (and cyclosporin A), and FK-506, or any combination thereof.

The primary immunosuppressive agent presently used for inhibiting rejection in the allograft transplantation of organs in man is cyclosporine (Sandimmune®). Cyclosporine is a cyclic polypeptide consisting of 11 amino acids. The intravenous injectable formulation of Sandimmune® (IV) is a sterile ampul containing, per ml, 50 mg of cyclosporine, 650 mg of Cremophor® EL and alcohol Ph Helv. (32.9% by volume) (under nitrogen). For administration this mixture is diluted further with 0.9 % Sodium Chloride Injection or 5% Dextrose Injection before use. (Physicians' Desk Reference, 45th ed., 1991, pp. 1962-64, Medical Economics Company, Inc.) The

macrolide molecule designated FK506, which has certain structural similarities to rapamycin, is also currently undergoing clinical investigation for inhibiting rejection in allograft organ transplantation in man. FK506 is isolated from *Streptomyces tsuskubaensis* and is described in U.S. Patent No. 4,894,366 to Okuhara et al., issued January 16, 1990 R. Venkataramanan et al., in Transplantation Proceedings, 22, No. 1, Suppl., 1 pp 52-56 (February 1990), report that the intravenous injectable formulation of FK506 is provided as a 10 mg/ml solution of FK506 in polyoxyethylated castor oil (HCO-60, a surfactant) and alcohol. The intravenous preparation must be diluted with saline or dextrose and administered as an infusion for 1 to 2 hours.

The Physicians' Desk Reference (45th ed., 1991, p. 2119, Medical Economics Company, Inc.) lists cyclosporine under the Sandimmune® tradename as available in 25 mg and 100 mg strength capsules and as an oral solution in 50 ml bottles. The 25 mg capsules contain 25 mg cyclosporine, USP, and alcohol, USP dehydrated, at a maximum of 12.7% by volume. The 100 mg capsules contain cyclosporine, USP, 100 mg and alcohol, USP dehydrated, at a maximum 12.7% by volume. Inactive ingredients in the oral capsules are corn oil, gelatin, glycerol, Labrafil M 2125 CS (polyoxyethylated glycolysed glycerides), red iron oxide, sorbitol, titanium dioxide, and other ingredients. The oral solution is available in 50 mg bottles containing cyclosporine, USP, 100 mg and Ph. Helv. alcohol at 12.5% by volume dissolved in olive oil, Ph. Helv./Labrafil M 1944 CS (polyoxyethylated oleic glycerides) vehicle which must be diluted further with milk, chocolate milk or orange juice before oral administration.

Azathioprine (available from Burroughs Wellcome Co., Research Triangle Park, N.C., under the tradename Imuran®) is another orally administered immunosuppressive agent prescribed alone or in conjunction with other immunosuppressive agents. The Physicians' Desk Reference (45th ed., 1991, pp. 785-787, Medical Economics Company, Inc.) lists azathioprine as 6-[1-methyl-4-nitroimidazol-5-yl)thio]purine, which is provided for oral administration in scored tablets containing 50 mg azathioprine and the inactive ingredients lactose, magnesium stearate, potato starch, povidone, and stearic acid.

Methods of drug delivery are designed to deliver an acceptable dosage of the medication to the patient. In the case of oral formulations, it is highly desirable to provide a dosage form which meets this criteria and which can be effectively administered, preferably self-administered, in either clinical or non-clinical situations. The present invention concerns formulations useful in the oral administration of rapamycin. Rapamycin has been shown to possess immunosuppressive, antifungal and antiinflammatory activity in vivo and to inhibit thymocyte proliferation in vitro. Therefore, these formulations are useful in the treatment of Candida albicans infections, diseases of inflammation and transplant rejection autoimmune diseases, including lupus, rheumatoid arthritis, diabetes melitus, multiple sclerosis, etc.

Because the formulations disclosed herein contain rapamycin, they are considered to have antitumor, antifungal and antiproliferative activities. As such, the formulations of this invention are useful in the treatment of transplantation rejection, such as heart, kidney, liver, bone marrow and skin transplants; autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis and multiple sclerosis; diseases of inflammation such as psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease and eye uveitis; solid tumors; fungal infections; and hyperproliferative vascular diseases, such as restenosis. The present invention, therefore, also provides formulations useful for inducing immunosuppression in a mammal in such need. Such inducements would comprise administering to said mammal an immunosuppressive amount of one or more of the formulations discussed herein.

The present formulations comprise a rapamycin solution containing an organic solvent and phospholipid such as lecithin.

The formulations of the present invention may be produced as a one component, ready to use solution of rapamycin in a non-aqueous system consisting of a solvent and phospholipid to produce an acceptable dosage form for chronic use in association with immunosuppressant therapy, as well as antitumor, antifungal and antiproliferative activities. The one component system may be adjusted to eliminate the solvent in cases where the drug concentration can be solubilized in the remaining ingredients. The invention may also be produced alternatively as a two component system either comprised of a dry component fill of 100% rapamycin and diluent or a

drug concentrate and diluent. Other filler materials, such as lactose or mannitol, may be used as a portion of the dry component of such systems.

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives or components or integers.

Accordingly this invention provides a composition of matter containing from about 0.01 gram to about 10.0 grams of rapamycin per 100 ml of composition, and a solvent system, said solvent system comprising from about 0.05 to about 10% by volume of a surfactant, from 0 to about 25% by volume of inert solvent and from about 65 to 99.95% by volume of phospholipid solution in which phospholipid in said solution is from about 40% to about 75% by weight.

In a first preferred aspect this invention provides a composition of matter as defined above comprising about 0.01 to about 10 grams of rapamycin per 100 mls of composition and a solvent system comprising one or more of the following:

a) about 0.01 to about 20% by volume of an inert solvent; preferably 0.5 to about 20%; most preferably about 1 to 10%; and/or

b) about 0.1 to about 10% by volume of surfactant; preferably about 0.5 to about 8%; most preferably about 1 to about 5%; and/or

c) about 65 to about 99.8% by volume of a phospholipid solution; preferably 72 to about 99.0% ; most preferably about 85 to about 98%

In a second preferred aspect this invention provides a composition of matter comprising from about 0.01 to about 1.0 gram of rapamycin per 100 mls and a solvent system comprising one or more of the following:

a) about 0.05% to about 10% volume of surfactant, preferably about 0.1% to about 5% by volume, most preferably 0.5 to about 5% by volume, and/or

b) about 90 to about 99.95% by volume of a phospholipid solution, preferably about 95 to about 99.9% by volume, most preferably about 95 to about 98.5% of a phospholipid solution.

It is further preferred that the phospholipid solution used comprises about 40% to about 60% by weight of phospholipid, most preferably about 50% by weight. The preferred phospholipid is lecithin. A preferred solvent for the phospholipid solution is propylene glycol but other solvents may be used as described herein.

In general, the formulations of the first aspect of this invention concern combinations of a) rapamycin, b) surfactant, c) N,N-dimethylacetamide (DMA) or other inert solvent and d) phospholipid e.g. lecithin in the following ranges (per 100 ml formulation):

a) rapamycin at a concentration of from about 0.01 to about 10.0 grams per 100 ml;

b) surfactant at a concentration of from about 0.1 to about 10.0 ml per 100 ml;

c) DMA at a concentration of from about 0.1 to about 25 ml per 100 ml; and

d) from about 65 to about 99.8 ml per 100 ml of a lecithin or phospholipid solution containing from 40 to 60 percent lecithin or phospholipid in suitable solvent.

More preferred formulations of the first aspect of the present invention include those combinations having the following ranges of materials:

a) rapamycin at a concentration of from about 0.05 to about 5.0 grams per 100 ml;

b) surfactant at a concentration of from about 0.5 to about 8.0 ml per 100 ml;

c) DMA at a concentration of from about 0.5 to about 20 ml per 100 ml; and

d) from about 72 to about 99.0 ml per 100 ml of a lecithin or phospholipid solution containing from 40 to 60 percent lecithin or phospholipid in suitable solvent.

The most preferred formulations of the first aspect of this invention include those having the following ranges of concentrations:

a) rapamycin at a concentration of from about 0.10 to about 1.0 gram per 100 ml;

b) surfactant at a concentration of from about 1.0 to about 5.0 ml per 100 ml;

c) DMA at a concentration of from about 1.0 to about 10 ml per 100 ml; and

d) from about 85 to about 98 ml per 100 ml of a lecithin or phospholipid solution containing from 40 to 60 percent lecithin or phospholipid in suitable solvent.

In general, the formulations or compositions of the second aspect of the present invention include those comprising combinations of a) rapamycin, b) surfactant, and c) lecithin or phospholipid in the following concentrations (per 100 ml formulation);

a) rapamycin at a concentration of from about 0.01 grams to about 1.0 gram per 100 ml;

b) Surfactant at a concentration of from about 0.05 ml to about 10 ml per 100 ml; and

c) from about 90 to about 99.95 ml per 100 ml of a lecithin or a phosholipid solution containing from about 40 to about 60 percent of lecithin or phospholipid in a suitable solvent.

More preferred formulations of the second aspect of the present invention include those having the following concentration (per 100 ml formulation):

a) rapamycin at a concentration of from about 0.03 grams to about 0.8 grams per 100 ml;

b) Surfactant at a concentration of from about 0.10 ml to about 5 ml per 100 ml; and

c) from about 95 to about 99.9 ml per 100 ml of a lecithin or a phosholipid solution containing from about 40 to about 60 percent of lecithin or phospholipid in a suitable solvent.

A most preferred formulation of the second aspect of the present invention includes those formulations having concentrations of ingredients within the following ranges:

a) rapamycin at a concentration of from about 0.05 grams to about 0.5 grams per 100 ml;

b) Surfactant at a concentration of from about 0.5 ml to about 5 ml per 100 ml; and

c) from about 95 to about 99.5 ml (preferably 95 to 98.5 ml) per 100 ml of a lecithin or a phosholipid solution containing from about 40 to about 60 percent of lecithin or phospholipid in a suitable solvent.

An example of a composition of matter of this invention contains about 1.0 gram of rapamycin in a solvent system comprising about 1% by volume of surfactant and about 99% by volume of a phospholipid solution containing 50% by weight of phospholipid.

Further examples of compositions of matter for oral administration comprise, per 100 ml of the composition,

a) a first 20 ml component of 2500 mg of rapamycin in N,N-dimethylacetamide; and

b) a second component of from 0.05 gm/ml to 0.07 gm/ml of surfactant in lecithin, the second component being added to the first 20 ml component to complete a 100 ml composition volume.

Yet a further example is a composition of matter for oral administration comprising, per 100 ml composition, 2.5 grams of rapamycin, 5.0 ml of surfactant, about 13 ml of absolute ethanol, and a 50% lecithin solution q.s to 100 ml.

The examples provided below list a number of solvents that are useful as inert solvents or as solvent in the phospholipid solution for the formulations of the present invention. Alternate solvents that can be used include, but are not limited to, dimethylacetamide, ethanol, dimethylformamide, glycerin, t-butanol polythylene glycol and propylene glycol. The amounts of the solvents can be raised in conjunction with the drug concentration(s) As another alternative, the amounts of the solvents can be

reduced in conjunction with the drug concentration and, if drug solubility permits, the lecithin solution alone can act as the solvent.

Examples of surfactants in the compositions of matter according to the present invention are one or more of the following:
polyoxyethylene sorbitol esters, polyoxyethylated fatty acids, polyoxyethylated fatty alcohols and polyoxyethylated glycerin hydroxy fatty esters.

Particular surfactants that may be used with the present formulations include, but are not limited to, Polysorbate 20 (polyoxyethylene 20 sorbitan monolaurate), Polysorbate 60, Span 80® Sorbitan Oleate, a product of ICI Americas, Wilmington, DE, the Cremophor® surfactants produced by the BASF Corporation, Parsippany, NJ, and Polysorbate 80, which is defined by the Merck Index, 11th Edition, published by Merck & Co., Inc., Copyright 1989, on page 1254 as Sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives, polyoxyethylene (20) sorbitan mono-oleate, Sorbitan mono-oleate polyoxyethylene, Sorlate, Tween 80, among others, and indicates an oleate ester of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides. Polysorbate 80 is the surfactant preferred with the present invention.

A number of lecithin or phospholipid solutions may be used in the present formulations. Lecithin is a general term for phosphatidylcholine or a mixture of various diglycerides of stearic, palmitic, and oleic acids, linked to the choline ester of phosphoric acid. Various types of lecithin or lecithin sourced products (such as separated phospholipids), either alone or mixed with various solvents wherein the lecithin comprises about 40-75%, e.g. about 40-60%, can be used as the final ingredient of the formulations mentioned above. These lecithin ingredients can include, for example, Alcolec® lecithin, produced by the American Lecithin Company, Danbury, CT, Phosal 50 PG propylene glycol and lecithin, Phosal 50 MCT phosphatidylcholine and medium chained triglycerides, and Phospholipan 90® lecithin, all of which are produced by Nattermann Phospholipid GMBH, Colone, Germany, the Centrophil® and Centrophase® lecithins produced by Central Soya, Fort Wayne, IN. It is preferred that the phospholipid solutions used in the present formulation have at least a 50% concentration of phospholipid. More particularly, it is preferred that the lecithin products or solutions used with the present formulations have at least 50%

phosphatidylcholine. It is also preferred that the phospholipid solution comprise a phospholipid in propylene glycol.

The dosage requirements may vary the severity of the symptoms presented and the particular subject being treated. Projected daily oral dosages of the compounds of this invention would be 0.005 - 75 mg/kg, preferably between 0.01 - 50 mg/kg, and more preferably between 0.05 - 10 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached. Precise dosages will be determined by the administering physician based on experience with the individual subject treated. In general, the formulations of this invention are most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects.

The present formulations may be administered to the patient by the means generally used for oral liquid medications. They may be taken, by themselves, or they may be dispersed in a liquid, such as water or juices. The formulations may also be capsulized, such as in starch capsules or soft elastic gelatin capsules. Rapamycin oral may be dispersed into water for dosing in the range of about 1 part of formula into about 9 parts water downward to about 1 part of formula into about 499 parts water by mixing for a minimum of about 60 seconds. This dispersion may be used over about a 1 hour period with mixing prior to dosing.

It is contemplated that when the formulations of this invention are used as an immunosuppressive or antiinflammatory agent, they can be administered in conjunction with one or more other immunoregulatory agents. Such other antirejection chemotherapeutic agents include, but are not limited to azathioprine, corticosteroids, such as prednisone and methylprednisolone, cyclophosphamide, cyclosporin A, FK-506, OKT-3, and ATG. By combining one or more of the formulations of the present invention with such other drugs or agents for inducing immunosuppression or treating inflammatory conditions, lesser amounts of each of the agents may be required to achieve the desired effect. The basis for such combination therapy was established by Stepkowski whose results showed that the use of a combination of rapamycin and

cyclosporin A at subtherapeutic doses significantly prolonged heart allograft survival time. [Transplantation Proc. 23:507 (1991)].

This invention also provides a process for preparing a composition of matter which comprises dissolving rapamycin in one or more components of a solvent system comprising from about 0.05 to about 10% by volume of surfactant, from 0 to about 25% by volume of inert solvent and from about 65 to 99.95% by volume of phospholipid solution in which phospholipid in said solution is from about 40% to about 75% by weight such that the concentration of rapamycin in the total composition is from about 0.01 to about 10.0 grams per 100ml.

It is also understood that the present formulations may be used with other ingredients used with conventional oral formulations such as, but not limited to, flavor enhancers, coloring agents, adjuvants, antifungal agents, antibacterial agents, etc.

The following non-limiting examples and comparative examples are provided to illustrate the effectiveness of the more preferred embodiments of the present invention.

EXAMPLES

EXAMPLE 1

The following Example 1 demonstrates an oral rapamycin formulation having a concentration of rapamycin which is 50mg/kg.

A. Formula:

Ingredients		Amount
Rapamycin @ 100%	up to	5.0 gm
Polysorbate 80, NF		5.0 ml or 5.4 gm
N,N-dimethylacetamide		20.0 ml or 18.7 gm
Phosal 50 PG[1]	q.s.	100 ml or 99.6 gm

[1]Nattermann brand of lecithin and propylene glycol

Manufacturing Directions:

1. Weigh the rapamycin into a suitable container.
2. Add the N,N-dimethylacetamide to the container in Step #1. Mix until dissolved.
3. Add the Polysorbate 80 to the container in Step #2. Mix until uniform.
4. Adjust to the final volume with Phosal 50 PG® lecithin and propylene glycol.
5. Mix until uniform

Two Cynomolgus monkeys, listed below as A and B, were administered the above formulation at a dose of 50 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing.

Rapamycin Concentration in Monkey Serum Dosed Orally with 50 mg/kg

Time	A	B
0	BDL	BDL
1 hr	0.017	0.035
2 hr	0.037	0.166
3 hr	0.062	0.078
4 hr	0.215	0.115
6 hr	0.262	0.050
9 hr	0.103	0.010
12 hr	0.018	BDL

BDL = Below detection limit (detection limit ~ or equal to 0.006 µg/ml)

EXAMPLE 2

The following Example 2 provides a oral formulation having a rapamycin concentration of 125 mg/ml, as well as the procedure for its preparation. The first set of ingredients and procedures provided demonstrate the production of an oral rapamycin concentrate. The second set of ingredients and procedures provided demonstrate a diluent which may be used with the rapamycin concentrate.

Rapamycin Oral Concentration at 125 mg/ml in DMA

Formula:

Ingredients		Amount
Rapamycin @ 100%		12.5 gm
Dimethylacetamide (DMA)	q.s.	100 ml

Procedure:

1. Weigh 12.5 g of rapamycin into a suitably calibrated container.
2. Q.S. to 100 ml with DMA.
3. Mix until a clear solution is formed.
4. Store rapamycin concentrate in all glass container or in a flint glass vial stoppered with a Teflon barrier faced stopper.

The following Diluent No. 1 is used in the oral rapamycin formula (rapamycin at 25 mg/ml) which follows:

Diluent No. 1 for Oral Rapamycin

Formula:

Ingredients		Amount
Polysorbate 80		6.69 gm
Centrophil W[1]	q.s.	100 ml

[1]Central Soya brand of lecithin

Procedure:

1. Add 6.69 grams of Polysorbate 80 to a suitable container
2. Q.S. to 100 ml with Centrophil W® lecithin.
3. Mix until homogeneous.
4. Diluent for oral rapamycin can be stored in an all glass container or in a flint glass vial stoppered with a Teflon barrier faced stopper at room temperature.

Rapamycin Oral at 25 mg/ml

Formula:

Ingredients	Amount
Rapamycin Oral Concentrate @ 125 mg/ml	20 ml
Diluent for Oral Rapamycin	q.s. 100 ml

Procedure:

1. Place 20 ml of rapamycin oral concentrate into a container.
2. Q.S. to 100 ml with diluent for oral rapamycin.
3. Mix until homogeneous.
4. This rapamycin formula can be stored in an all glass container or in a flint glass vial stoppered with a Teflon barrier faced stopper.

Four Cynomolgus monkeys, listed below as A-D, were administered the above formulation at a dose of 50 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing.

Rapamycin Concentration in Monkey Serum Dosed Orally with 50 mg/kg

	Rapamycin Concentration (μg/ml)			
	Monkey No.			
Time	A	B	C	D
0	BDL	BDL	BDL	BDL
1 hr	0.008	0.786	0.078	0.053
2 hr	0.020	0.129	0.066	0.013
3 hr	0.026	0.077	0.101	0.022
4 hr	0.104	0.036	>0.200	0.057
6 hr	QNS	0.029	>0.200	0.117
9 hr	0.113	0.012	>0.200	0.031
12 hr	0.022	0.005	0.050	0.005

QNS = Quantity not sufficient.
BDL = Below detection limit (detection limit ~ or equal to μg/ml)

The procedural steps for formulation and storage of the 5 mg/ml oral rapamycin formulation are the same as those listed in Example 1, as are the alternative order of addition of ingredients and the methods of comminution.

EXAMPLE 3

Rapamycin Oral at 1 mg/ml

A rapamycin oral formulation at a concentration of 1 mg/ml can be formulated from the following active and inactive ingredients by the procedural steps which follow:

Active Ingredient:	Conc.	Input	Batch Formula 10,000 bottles
Rapamycin @ 100%	1.00 mg/ml	0.025 g	0.250 kg
Inactive Ingredients:			
Polysorbate 80, NF	10.8 mg/ml	0.270 g	2.700 kg
Phosal 50 PG® propylene glycol and lecithin q.s. ad	1.00 ml	25.0 ml	250.0 L
or	1.005 gm	or 25.125 g	251.25 kg

Density of the Final Formulation - 1.005 g/ml

If the potency of the rapamycin is less than 100%, the input must be adjusted to achieve the claimed potency.

Method of Manufacture

Procedure:

1. Weigh the rapamycin into a suitable container.
2. Add the Polysorbate 80 to the container in step #1
3. Adjust to the final volume with Phosal 50 PG.
4. Mix until the rapamycin is dissolved.
5. Fill 25 ml ± 1.25 ml (25.125 g ± 1.256 g) into each one ounce amber glass bottle. It is preferable to seal with a child resistant cap.

For improved wettability and ease of solution, an alternative order of addition of the ingredients and amounts presented above is as follows:

1. Polysorbate 80.
2. A portion of the Phosal 50 PG propylene glycol and lecithin.
3. Rapamycin.
4. The remaining Phosal 50 PG propylene glycol and lecithin.

The rapamycin in these formulations may also be comminuted by use of a mill or mortar and pestle and passed through an 80 mesh screen.

EXAMPLE 4

Rapamycin Oral at 5 mg/ml

A rapamycin oral formulation at a concentration of 5 mg/ml can be formulated from the following active and inactive ingredients by the procedural steps which follow:

Active Ingredient:	Conc.	Input	Batch Formula 10,000 bottles
Rapamycin @ 100%	5.00 mg	0.125 g	1.250 kg
Inactive Ingredients:			
Polysorbate 80, NF	10.8 mg	0.270 g	2.70 kg
Phosal 50 PG propylene glycol and lecithin q.s. ad	1.00 ml	25.0 ml	250.0 L
or	1.005 gm	or 25.125 g	or 251.25 kg

Density of the Final Formulation - 1.005 g/ml.

If the potency of the rapamycin is less than 100%, the input must be adjusted to give the claimed potency.

The procedural steps for formulation and storage of the 5 mg/ml oral rapamycin formulation are the same as those listed in Example 3, as are the alternative order of addition of ingredients and the methods of comminution.

EXAMPLE 5

The formulation of this Example was produced using the ingredients which follow and the methods indicated below:

Ingredients	Amount
Rapamycin @ 100% up to	1.0 gm
Polysorbate 80, NF	1.0 ml or 1.08 gm
Phosal 50 PG lecithin and propylene glycol q.s.	100 ml or 100.5 gm

Method of Formulation

1. Weigh the rapamycin into a suitable container.
2. Add the Polysorbate 80 into the container of Step #1.
3. Adjust to the final volume with Phosal 50 PG® propylene glycol and lecithin.
4. Mix until a solution results.

Alternatively, this formula can be packaged in a suitable container or encapsulated into a capsule.

EXAMPLE 6

The oral formulations of this invention, such as those disclosed in the Examples above, may also be prepared in encapsulated forms, such as formulations within starch capsules. The following procedure describes a method which may be utilized to prepare such encapsulated formulations.

Procedure:

1) Add to a container, NF, the Polysorbate 80.

2) Add to the Polysorbate 80 of Step #1 80% of the the required Phosal 50 PG.

3) Weigh the rapamycin component of the formulation into the container of Step #2.

4) Adjust to the final formulation weight with Phosal 50 PG.

5) Establish a nitrogen atmosphere over the formulation and maintain until the capsules are filled.

6) Mix the formulation until the rapamycin is dissolved.

7) Pass the formulation solution through a particulate (such as a 100 mesh screen) or scintered glass filter.

8) Fill 0.50 ml of the Step #7 material into capsule shells using an automatic syringe dispensing unit and seal the capsule.

9) Package the filled capsules upon completion of encapsulation. An example of a preferred package is a conventional blister package with a perforable metal foil backing.

10) Store the finished encapsulated product at refridgerated conditions (2-8°C) protected from light.

The primary capsule sealant for the starch capsule may be a 5% Dextrin, NF, aqueous solution. It is preferable to heat purified water to 50-60° C prior to compounding to facilitate dissolution of the Dextrin. Prior to use it is also preferable to filter the the Dextrin solution through a suitable particulate filter.

Bioavailability

a) Cynomolgus monkeys were administered a starch encapsulated formulation of Example 5, above, at a dose of 0.25 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing:

Rapamycin Concentration in Monkey Serum
Dosed Orally as a Dispersion of 0.25mg/kg

Rapamycin Concentrate (μg/ml)

Time (hrs)	A	B	C	D	E	F
0	0.000 0.000	0.000	0.000	0.000	0.000	
.25	0.012 0.000	0.001	0.005	0.000	0.000	
.50	0.014 0.003	0.000	0.024	0.004	0.000	
1	0.011 0.004	0.002	0.021	0.006	0.003	
2	0.005 0.003	0.019	0.008	0.004	0.007	
4	0.002 0.002	0.006	0.007	0.003	0.006	
8	0.002 0.001	0.004	0.005	0.003	0.002	
12	0.001 0.001	0.002	0.003	0.002	0.001	
24	0.001 0.001	0.002	0.001	0.001	0.002	
36	0.000 0.000	0.002	0.001	0.001	0.000	

Rapamycin Concentration in Monkey Serum
Dosed Orally in Starch Capsules 0.25mg/kg

Rapamycin Concentrate (µg/kg)

Time (hrs)	A	B	C	D	E	F
0	0.000 0.000	0.000	0.000	0.000	0.000	
.25	0.000 0.000	0.000	0.000	0.000	0.000	
.50	0.000 0.000	0.000	0.005	0.000	0.005	
1	0.029 0.000	0.004	0.026	-------	0.008	
2	0.011 0.004	0.019	0.032	0.000	0.011	
4	0.007 0.002	0.009	0.011	0.002	0.007	
8	0.004 0.002	0.003	0.004	0.002	0.005	
12	0.002 0.001	0.001	-------	0.001	0.002	
24	0.001 0.000	0.000	0.002	0.001	0.001	
36	0.000 0.000	0.000	0.000	0.000	0.000	

Rapamycin Concentration in Monkey Serum
Dosed Orally in SEG Capsules at 0.25 mg/kg

Time (hrs)	A	B	C	D	E	F
0	0.000 0.000	0.000	0.000	0.000	0.000	
.25	0.005 0.002	0.002	0.001	0.001	0.001	
.50	0.001 0.001	0.001	0.002	0.002	0.001	
1	0.043 0.012	0.022	0.019	0.002	0.003	
2	0.027 0.008	0.030	0.019	0.002	0.010	
4	0.012 0.006	0.012	0.015	0.009	0.011	
8	0.008 0.003	0.006	0.009	0.004	0.006	
12	0.008 0.002	0.004	0.006	0.002	0.005	
24	0.006 0.001	0.003	0.005	0.001	0.002	
36	0.002 0.001	0.001	0.002	0.001	0.002	

b) 3 mg starch encapsulated formulations containing rapamycin at a concentration of 6 mg/ml, prepared as described above, were administered to 14 healthy male human volunteers between the ages of 18 and 45, from whom blood samples were drawn at the time intervals indicated in the table below. The rapamycin blood samples were assayed for whole blood rapamycin concentration using a validated (ESP)-HPLC-MS method.

Time Interval Following Administration (Hours)	Blood Concentration (conc. = ng/ml)
0.33	0.41
0.67	6.53
1	8.57
2	8.27
3	5.54
4	3.96
5	3.10
8	1.93
12	1.47
18	1.05
24	0.80
48	0.54

EXAMPLE 7

Formula		Ingredients
Rapamycin @ 100%	up to	2.5 grams
Polysorbate 80, NF		5.0 ml or 5.4 gm
Absolute Ethanol		12.67 ml or 10.0 gm
Phosal 50 PG lecithin and propylene glycol	q.s.	100 ml

This formulation can be produced by the following steps:

1. Weigh the rapamycin into a suitable container
2. Add the absolute ethanol to the container in Step #1. Mix until dissolved.
3. Add the polysorbate 80 to the container in Step #2. Mix until uniform.
4. Add Phosal 50 PG lecithin and propylene glycol to adjust to the final volume.
5. Mix until uniform.

Alternatively, this formula can be packaged in a suitable container or encapsulated into a capsule.

Cynomolgus monkeys were administered the formulation above at a dose of 0.25 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing.

Rapamycin Concentration in Monkey Serum Dosed Orally in a Dispersion at 0.25 mg/kg

Rapamycin Concentration (µg/ml)
Monkey No.

Time (hr)	A	B	C	D	E	F
0	0.000	0.000	0.000	0.000	0.000	0.000
.25	---	0.025	0.007	0.010	0.007	0.003
.50	0.008	0.030	0.027	0.004	0.016	0.012
1	0.050	0.022	0.051	0.006	0.051	0.011
2	0.026	0.026	0.026	0.019	0.025	0.006
4	0.008	0.011	0.020	0.005	0.018	0.006
8	0.008	0.004	0.009	0.003	0.011	0.003
12	0.004	0.002	0.006	0.005	0.007	0.003
24	0.002	---	0.004	0.002	0.004	0.001
36	0.000	0.003	0.003	0.001	0.003	0.002

Rapamycin Concentration in Monkey Serum Dosed Orally in SEG Capsules at 0.25 mg/kg

Rapamycin Concentration (µg/ml)
Monkey No.

Time (hr)	A	B	C	D	E	F
0	0.000	0.000	0.000	0.000	0.000	0.000
.25	0.000	0.000	0.000	0.000	0.001	0.001
.50	0.000	0.000	0.000	0.000	0.010	0.006
1	0.030	0.013	0.001	0.000	0.019	0.005
2	0.014	0.024	0.014	0.002	0.014	0.005
4	0.013	0.011	0.003	0.006	0.015	0.004
8	0.007	0.004	0.002	0.002	0.007	0.002
12	0.005	0.003	0.001	0.001	0.006	0.001
24	0.003	0.001	0.001	0.001	0.003	0.001
36	0.002	0.001	0.001	0.000	0.001	0.000

COMPARATIVE EXAMPLES

COMPARATIVE EXAMPLE 1

The following traditional formulation approaches applied to rapamycin are provided as a comparison to those of the present invention. The ingredients and manufacturing directions for Diluent No. 2, below, are used to create the diluent in the comparative oral formulation (Rapamycin Oral Suspension at 50 mg/ml) which follows:

Diluent for Rapamycin Suspension

Ingredients	Amount
Polysorbate 80, NF	5.0 ml
0.5 M Citric Acid (pH 4)	q.s. 100 ml

Manufacturing Directions:

1. Prepare a 0.5 M citric acid solution.
2. Adjust the pH of the solution in Step #1 to 4.0 using 50% w/w NaOH.
3. Place the Polysorbate 80 into a suitable container.
4. QS to 100 ml with solution from step #2..
5. Mix until uniform.

Rapamycin Oral Suspension at 50 mg/ml

Ingredients	Amount
Rapamycin Micronized @ 100%	up to 5.0 gm
Diluent for Rapamycin Oral Suspension	q.s. 100 ml

Manufacturing Directions:

1. Weigh the rapamycin into a suitable container.
2. QS with the diluent for rapamycin
3. Mix until uniform.

Three Cynomolgus monkeys, listed below as A-C, were administered the above formulation at a dose of 50 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing.

Rapamycin Concentration in Monkey Serum Dosed Orally with 50 mg/kg Rapamycin Oral Suspension

	Rapamycin Concentration (µg/ml)		
	Monkey No.		
Time	A	B	C
0	BDL	BDL	BDL
1 hr	BDL	BDL	BDL
2 hr	BDL	BDL	BDL
3 hr	BDL	BDL	BDL
4 hr	BDL	BDL	BDL
6 hr	BDL	BDL	BDL
9 hr	BDL	BDL	BDL
12 hr	BDL	BDL	BDL

BDL = Below detection limit (detection limit ~ or equal to 0.006 µg/ml).

COMPARATIVE EXAMPLE 2

The following ingredients and procedural steps demonstrate the production of another traditional approach which has been applied to form an oral rapamycin solution, which is provided for comparison with the present invention.

Rapamycin Oral Solution at 50 mg/ml

Formula:

Ingredients	Amount
Rapamycin @ 100%	5.0 gm
Dimethylacetamide	10.0 gm
Absolute Ethanol	10.0 gm
Miglyol 812	q.s. 100 ml

Procedure:

1. Place rapamycin into a suitable container.
2. Add the dimethylacetamide and ethanol to the container in Step #1 and mix until a solution results.
3. QS with Miglyol 812 and mix until uniform.
4. (Alternative Step) Filter sample through a 0.2 micron Teflon filter.

Three Cynomolgus monkeys, listed below as A-C, were administered the above formulation at a dose of 50 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing.

Rapamycin Concentration in Monkey Serum Dosed Orally with 50 mg/kg Rapamycin Oral Solution

	Rapamycin Concentration (µg/ml)		
	Monkey No.		
Time	A	B	C
0	BDL	BDL	BDL
1 hr	BDL	BDL	BDL
2 hr	BDL	BDL	BDL
3 hr	BDL	BDL	BDL
4 hr	BDL	BDL	BDL
6 hr	BDL	BDL	BDL
9 hr	BDL	BDL	BDL
12 hr	BDL	BDL	BDL

BDL = Below detection limit (detection limit ~ or equal to 0.006 µg/ml).

COMPARATIVE EXAMPLE 3

Rapamycin Oral Emulsion at 50 mg/ml

Formula:

Ingredients	Amount
Rapamycin @ 100%	5.0 gm
Dimethylacetamide	10 ml
Olive Oil	q.s. 100 ml

Procedure:

1. Place the rapamycin into a suitable container.
2. Add the dimethylacetamide to the container in Step #1 and mix until clear.
3. QS with olive oil and mix until homogenous.

Three Cynomolgus monkeys, listed below as A-C, were administered the above formulation at a dose of 50 mg/kg of rapamycin and the following serum concentrations were determined at the indicated time after dosing.

Rapamycin Concentration in Monkey Serum Dosed
Orally with 50 mg/kg Rapamycin Oral Emulsion

	Rapamycin Concentration (µg/ml)		
Time	A	B	C
0	BDL	BDL	BDL
20 min	BDL	BDL	BDL
40 min	BDL	BDL	BDL
80 min	BDL	BDL	BDL
3 hr	BDL	BDL	BDL
6 hr	BDL	0.110*	BDL
12 hr	BDL	BDL	BDL
24 hr	BDL	BDL	BDL

BDL = Below detection limit (detection limit ~ or equal to 0.006 µg/ml).

*assay result obtained from test lab appears aberent

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition of matter for oral administration containing from 0.01 gram to 10.0 grams of rapamycin per 100 ml of composition, and a solvent system, said solvent system comprising from 0.05 to 10% by volume of surfactant, from 0 to 25% by volume of inert solvent and from 65 to 99.95% by volume of phospholipid solution in which phospholipid in said solution is from 40% to 75% by weight.

2. A composition of matter according to Claim 1 containing from 0.01 to 10.0 grams of rapamycin per 100 ml of composition and a solvent system, said solvent system comprising 0.1 to 10% by volume of surfactant, from 0.1 to 25% by volume of inert solvent, and from 65 to 99.8% by volume of a phospholipid solution in which the phospholipid in said solution is from 40 to 60% by weight.

3. A composition of matter according to Claim 2 containing 0.05 to 5.0 grams of rapamycin per 100 ml of composition, and a solvent system comprising from 0.5 to 8.0% by volume of surfactant, from 0.5 to 20% by volume of inert solvent and from 72 to 99.0% by volume of phospholipid solution.

4. A composition of matter according to Claim 2 containing 0.1 to 1.0 grams of rapamycin per 100 ml of composition, and a solvent system comprising from 1.0 to 5.0% by volume of surfactant, from 1.0 to 10% by volume of an inert solvent and from 85 to 98.0% by volume of phospholipid solution.

5. A composition of matter according to Claim 1 containing from 0.01 to 1.0 gram of rapamycin per 100 ml of composition and a solvent system, said solvent system comprising from 0.05% to 10% by volume of surfactant, and from 90% to 99.95% by volume of a phospholipid solution wherein the phospholipid in said solution is from 40% to 60% by weight.

6. A composition of matter according to Claim 5 containing from 0.03 to 0.8 gram of rapamycin per 100 ml of composition and a solvent system, said solvent system comprising from 0.10% to 5% by volume of surfactant, and from 95% to 99.9% by volume of a phospholipid solution wherein the phospholipid in said solution is from 40% to 60% by weight.

7. A composition of matter according to Claim 6 containing from 0.05 to 0.5 gram of rapamycin per 100 ml of composition and a solvent system said solvent system comprising from 0.5% to 5% by volume of surfactant, and from 95% to 98.5% by volume of a phospholipid solution wherein the phospholipid in said solution is from 40% to 60% by weight.

8. A composition of matter according to Claim 5 containing about 1.0 gram of rapamycin in a solvent system comprising about 1% by volume of surfactant and about 99% by volume of a phospholipid solution containing 50% by weight of phospholipid.

9. A composition of matter according to any one of Claims 1 to 7 wherein the phospholipid is about 50% by weight of the phospholipid solution.

10. A composition of matter according to any one of Claims 1 to 9 wherein the phospholipid is lecithin.

11. A composition of matter according to any one of Claims 1 to 10 wherein the surfactant is selected from one of the following:
polyoxyethylene sorbitol esters, polyoxyethylated fatty acids, polyoxyethylated fatty alcohols and polyoxyethylated glycerin hydroxy fatty esters.

12. A composition of matter according to any one of Claims 1 to 11 wherein the inert solvent if present is selected from one or more of dimethylacetamide, ethanol, glycerin, dimethylformamide, t-butanol, polyethylene glycol and propylene glycol.

13. A composition of matter according to any one of Claims 1 to 12 wherein the solvent for the phospholipid solution is propylene glycol.

14. A composition of matter for oral administration comprising, per 100 ml of the composition,

a) a first 20 ml component of 2500 mg of rapamycin in N,N-dimethylacetamide; and

b) a second component of from 0.05 gm/ml to 0.07 gm/ml of surfactant in lecithin, the second component being added to the first 20 ml component to complete a 100 ml composition volume.

15. A composition of matter for oral administration comprising, per 100 ml composition, 2.5 grams of rapamycin, 5.0 ml of surfactant, about 13 ml of absolute ethanol, and a 50% lecithin solution q.s to 100 ml.

16 A composition of matter according to any one of Claims 1-15 when in unit dosage form.

17 A composition of matter according to Claim 16 in which the unit dosage form is a liquid filled capsule.

18. A process for preparing a composition of matter for oral administration which comprises dissolving rapamycin in one or more components of a solvent system comprising from 0.05 to 10% by volume of surfactant, from 0 to 25% by volume of inert solvent and from 65 to 99.95% by volume of phospholipid solution in which the phospholipid in said solution is from 40% to 75% by weight such that the concentration of rapamycin in the total composition is from 0.01 to 10.0 grams per 100ml.

19. A process for preparing a composition of matter according to Claim 16 which comprises dissolving rapamycin in one or more components of a solvent system comprising 0.1 to 10% by volume of surfactant, from 0.1 to 25% by volume of inert solvent, and from 65 to 99.8% by volume of a phospholipid solution in which the phospholipid in said solution is from 40 to 60% by weight such that the concentration of rapamycin in the total composition is from 0.01 to 10.0 grams per 100ml.

20. A process for preparing a composition of matter according to Claim 16 which comprises dissolving rapamycin in one or more components of a solvent system comprising from 0.05% to 10% by volume of surfactant, and from 90% to 99.95% by volume of a phospholipid solution wherein the phospholipid said solution is from 40% to 60% by weight such that the concentration of rapamycin in the total composition is from 0.01 to 1.0 grams per 100 ml.

21. A method of inducing immunosuppression in a mammal in need of such inducement comprising administering to said mammal an immunosuppressive amount of a composition of claim 1.

22. A method of treating transplantation rejection, host vs graft disease, autoimmune diseases, diseases of inflammation, solid tumours, fungal infections, adult T-cell leukemia/lymphomas or hyperproliferative vascular diseases in a mammal in need of one or more of such treatments comprising administering to said mammal an effective amount of a composition of claim 1.

23. A composition of matter according to claims 1, 14 or 15, substantially as hereinbefore described with reference to the Examples.

24. A process for preparing a composition of matter according to claims 18 or 19, substantially as hereinbefore described with reference to the Examples.

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ABSTRACT OF THE DISCLOSURE

The present invention comprises novel oral rapamycin formulations which contain about 0.01 to about 10.0 grams of rapamycin per each 100 ml of the formulation, and a solvent system comprising from about 0.1 to about 10 % by volume of surfactant, from about 0 to about 25 % by volume of inert solvent, and from about 65 to about 99.95 % by volume of a lecithin or phospholipid solution.