ORAL CYCLOSPORIN FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 08/620,021 filed March 21, 1996, which is a continuation-in-part of application Serial No. 08/519,689 filed August 25, 1995, which applications are herein incorporated by reference.

INTRODUCTION

Field of the Invention

The field of this invention is oral cyclosporin formulations.

Background

Despite efforts to avoid graft rejection through host-donor tissue type matching, in the majority of transplantation procedures where a donor organ is introduced into a host, immunosuppressive therapy is critical to the maintained viability of the donor organ in the host. A variety of immunosuppressive agents have been employed in transplantation procedures, including azathioprine, methotrexate, cyclophosphamide, FK-506, rapamycin and corticosteroids. Agents finding increased use in immunosuppressive therapy due to their preferential effect on T-cell mediated reactions are the cyclosporins.

Cyclosporins are a class of cyclic polypeptides consisting of eleven amino acids which are produced as a metabolite by the fungus species *Tolypocladium inflatum Gams*. Cyclosporins have been observed to reversibly inhibit immunocompetent lymphocytes, particularly T-lymphocytes, in the G_0 or G_1 phase of the cell cycle. Cyclosporins have also been observed to reversibly inhibit lymphokine production and release. Although a number of cyclosporins are known, Cyclosporin A is the most-widely used.

Use of Cyclosporin A has been reported to prolong the survival of allogeneic transplants involving skin, heart, kidney, pancreas, bone marrow, small intestine and lung. In allogeneic transplantations, Cyclosporin A has been shown to suppress humoral immunity and, to a greater extent, cell mediated immune reactions, including: allograft rejection, delayed hypersensitivity, experimental allergic encephalomyelitis, Freund's adjuvant arthritis, and graft vs. host disease. Although success has been realized with Cyclosporin A, following transplantation administration of the agent must be continued since the benefits of cyclosporin therapy are reversible and graft rejection occurs once administration of Cyclosporin A is discontinued.

Although cyclosporin formulations for both oral and intravenous administration have been developed, oral administration of cyclosporin is preferred because of the ease of administration and greater patient acceptance. Furthermore, intravenous administration of cyclosporin can result in anaphylactic reactions, a side effect not observed with oral formulations. Oral cyclosporin formulations which have been developed and are currently marketed include both soft gelatin capsule and solution formulations, both of which are sold under the trademarks SANDIMMUNE® and NEORAL™.

In using oral cyclosporin formulations in immunosuppressive therapy, both the care giver and manufacturer must be cognizant of many issues. With oral cyclosporin formulations, cyclosporin bioavailability can be limited because of cyclosporin's insolubility in water and the tendency of cyclosporin to precipitate in aqueous environments. In addition, the

concentration of cyclosporin present in oral formulations can be limited due to cyclosporin's hydrophobic nature. Finally, packaging and storage stability are an issue with oral formulations. For example, with soft gelatin capsule formulations of cyclosporin, air tight packaging must be employed, which is inconvenient due to bulkiness and high cost. Furthermore, cyclosporin formulations may be unstable at lower temperatures, as cyclosporin crystallization may occur.

Thus, desirable oral cyclosporin formulations would be formulations that address at least some of the above issues. Ideally, oral formulations would promote high bioavailability, comprise high concentrations of cyclosporin and would be amenable to preparation in both liquid and hard capsule forms.

Relevant Literature

Physician's Desk Reference (1994) pp 2071-2074 describes oral cyclosporin formulations currently sold under the trademark SANDIMMUNE®.

Oral cyclosporin formulations are also described in the NEORAL™ package insert, (1995) (Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey, 07936).

U.S. Patents of interest describing cyclosporins and derivatives thereof include: 4,220,641; 4,639,434; 4,289,851; and 4,384,996. U.S. Pat. No. 5,047,396 describes an intravenous preparation for administration of cyclosporin. U.S. Pat. Nos. 4,388,307; 4,970,076 and 4,990,337 describe the preparation of oral cyclosporin formulations.

The preparation of hard capsules for the oral delivery of pharmaceutical formulations is described in U.S. Pat. Nos. 4,822,618; 4,576,284; 5,120,710; and 4,894,235.

SUMMARY OF THE INVENTION

Oral cyclosporin formulations, and methods for their use in immunosuppressive therapy, are provided. In the subject formulations, cyclosporin is present in an orally acceptable vehicle comprising at least one alkanol solvent of from 2 to 3 carbon atoms in combination with at least one non-ionic surfactant. The subject formulations may further comprise one or more cosolvents, where cosolvents of interest are fatty acid esters and diols, preferably polyglycols. The cyclosporin formulations can be packaged as hard capsules. The formulations are substantially anhydrous which reduces precipitation of cyclosporin from the formulation and increases bioavailability.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 provides the cyclosporin peak concentration (C_{max}) achieved in rats for several oral formulations according to the subject invention, where the C_{max} is shown as a relative value compared to the C_{max} achieved with SANDIMMUNE® ORAL formulation (SO).

Fig. 2 provides the time at which C_{max} occurred (T_{max}) for each of formulations shown in Fig. 1, where T_{max} is provided as relative value compared to the T_{max} of SANDIMMUNE® ORAL formulation (SO).

Fig. 3 provides the relative area under the blood concentration-time curve (AUC) for each of the formulations shown in Fig. 1, where AUC is provided as a relative value compared to the AUC value for SANDIMMUNE® ORAL formulation (SO).

Fig. 4 provides the cyclosporin peak concentration (C_{max}) achieved in humans for several oral formulations according to the subject invention, as well as SANDIMMUNE® ORAL solution ("Sand" in the figure).

Fig. 5 provides the time at which C_{max} occurred (T_{max}) for each of formulations shown in Fig. 4.

Fig. 6 provides the area under the blood concentration-time curve (AUC) for each of the formulations shown in Fig. 4.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Oral cyclosporin formulations are provided which promote bioavailability and can be formulated as capsules, particularly hard capsules. In the subject formulations, cyclosporin is present in an orally acceptable vehicle comprising at least one alkanol solvent of from 2 to 3 carbon atoms in combination with at least one non-ionic surfactant. The subject formulations may further comprise at least one cosolvent, where cosolvents of interest include fatty acid esters and diols, preferably one or more polyglycols. Each of the components of the subject formulations are pharmaceutically acceptable. In addition to providing for high bioavailability, the subject formulations provide for reproducible cyclosporin absorption from one batch of a particular formulation to the next. The subject formulations find use in immunosuppressive therapy.

A number of cyclosporins are known in the art to exhibit immunosuppressive activity and may be delivered in the subject oral formulations. Cyclosporins that may be administered in the subject formulations include Cyclosporin A, Cyclosporin B, Cyclosporin C, Cyclosporin D and Cyclosporin G, as well as synthetic analogs thereof. *See* Merck Index (1989) 2759. The subject oral formulations are particularly suited for the delivery of Cyclosporin A. When delivered in the subject formulations, Cyclosporin A will be present in concentrations ranging from 50 to 150 mg/ml, usually 100 to 150 mg/ml, based on the volume of the vehicle component of the formulation.

The vehicle component of the subject formulations will include an alkanol solvent component, where the alkanol solvent component will comprise at least one alkanol and usually no more than three different alkanols, more usually no more than two different alkanols, where the alkanols will usually be from 2 to 3 carbon atoms, and from 1 to 2 hydroxy groups, such that there is no more than 1 hydroxy group per 1.5 carbon atoms. Suitable alkanols include ethanol and propylene glycol, preferably absolute ethanol. The total amount of alkanol solvent in the formulation will be at least about 1 % (v/v), usually at least about 3 % (v/v) and may be as high as 95 % (v/v), but will generally range from

about 5 to 75 % (v/v), usually from about 5 to 60 % (v/v), and more usually from about 10 to 60 % (v/v) of the formulation. When ethanol is present in the formulation as an alkanol solvent, the amount of ethanol may range from 5 to 25 % (v/v), usually from about 5 to 20 % (v/v) and more usually from about 10 to 25 % (v/v) of the formulation, while when propylene glycol is present as an alkanol solvent, the amount of propylene glycol in the subject formulation may range from about 5 to 90 % (v/v), usually from about 5 to 85 % (v/v), more usually from about 10 to 50 % (v/v) of the formulation.

Also present in the orally acceptable vehicle will be at least one non-ionic polyoxyalkylene surfactant, usually not more than two non-ionic polyoxyalkylene surfactants. The polyoxyalkylene surfactants will have a hydrophilic-lipophilic-balance (HLB) of from about 5 to 20, usually from about 8 to 16. Preferably, the non-ionic polyoxyalkylene surfactants employed in the subject formulations will be polyoxyethylene compounds. Polyoxyethylene compounds of interest include: ethoxylated alcohols, *i.e.* polyoxyethylene alcohols or ethoxylated fatty alcohols, where the alcohol moieties are generally of from 10 to 18, usually from 10 to 14 carbon atoms, as well as ether and ester substituents thereof; and polyoxyethylene derivatives of fatty acid partial esters, usually monoesters, of polyols of from 4 to 6 carbon atoms, usually 6 carbon atoms, where the polyols may be polyol anhydrides *e.g.* sorbitan. The fatty acid moieties of the subject surfactant will typically range from 10 to 18 carbon atoms. The number of ethyleneoxide groups will generally be in the range of 2 to 30, usually in the range from about 2 to 25. Preferred surfactants are polyoxyethylene (4) lauryl ether (BRIJ 30®) and polyoxyethylene (20) mono sorbitan mono-oleate (TWEEN 80®). The total amount of non-ionic surfactants present in the subject formulations will range from 5 to 80 %, usually from about 5 to 70 %, more usually from 5 to 65%, preferably from 5 to 60 % (v/v), more preferably from 20 to 75 % (v/v) and often 50 to 80 % of the formulation. Where TWEEN 80® is present in the formulation, it will usually be present in amounts ranging from 5 to 80 %, more usually from 5 to 70 %, preferably from 5 to 60 %

and more preferably from about 10 to 50 % (w/v) of the formulation. When BRIJ 30® is present in the subject formulation, it will usually be present in amounts ranging from 10 to 45 %, more usually from about 15 to 40 % (v/v) of the formulation.

The subject formulations may further comprise one or more cosolvents, usually not more than three different cosolvents, more usually not more than two different cosolvents, where suitable cosolvents include fatty acid esters and diols, where the cosolvent may be 100 % fatty acid ester, 100 % diol, or a combination thereof. In formulations comprising a cosolvent which is 100 % fatty acid ester or 100 % diol, different fatty acid esters or diols may be employed in combination. The total amount of cosolvent present in the formulation may range from about 20 to 80 % (v/v) and will usually range from about 25 to 75 % (v/v). When present in the formulation, the ratio of cosolvent to solvent in the subject formulations may range from about 1:1 to 15:1, but will usually range from about 1:1 to 13:1.

Fatty acid esters which may serve as cosolvents in the subject formulations are those fatty acid esters where the hydrocarbon chain of the fatty acid is from 12 to 18, usually 14 to 18 carbon atoms in length, where the fatty acid ester will be a mono-ester of a lower alkanol. Suitable fatty acid esters will generally comprise an even numbered fatty acid chain, where the hydrocarbon chain may be saturated or unsaturated, usually having not more than two sites of unsaturation. Fatty acids of interest will generally be of plant or mammalian origin and include palmitate, stearate, palmitoleate, linoleate, linolenate and the like, particularly myristate and oleate. The alcohol of the fatty acid mono-ester will be a lower alkanol of from 2 to 4 carbon atoms in length, usually 2 to 3 carbon atoms in length, with or without branches. Fatty acid esters of particular interest are isopropyl myristate and ethyl oleate. Isopropyl myristate, when present, will range from about 15 to 75 % (v/v), and ethyl oleate, when present, will range from about 15 to 75 % (v/v) of the total formulation. Usually the fatty acid ester will be present in an amount at least about equal (v/v) and up to 8 times the amount of surfactant in

the formulation, usually not greater than 5 times the amount of surfactant in the formulation (v/v). The fatty acid ester is preferably anhydrous.

Diols may also be present in the subject formulations, where the diols may be present in addition to, or in lieu of, the fatty acid ester cosolvent. The diols are preferably anhydrous. Diols of interest as cosolvents are generally liquids at physiologic temperatures and include diols of from 8 to 28 carbon atoms, usually 16 to 20 carbon atoms, where the diol may be a polyoxyalkylene diol, where alkylene is of from 2 to 3 carbon atoms, however, diols having significantly more carbon atoms also find use. Suitable diols for use as cosolvents may range from about 200 to 10,000 daltons, usually from about 300 to 10,000 daltons, more usually from 400 to 10,000 daltons, preferably 800 to 10,000 daltons. Diols of particular interest include polyethylene glycols, particularly polyethylene glycol 200 (PEG_{200}), polyethylene glycol 300 (PEG_{300}), polyethylene glycol 400 (PEG_{400}), polyethylene glycol 600 (PEG_{600}), polyethylene glycol 1000 (PEG_{1000}), polyethylene glycol 3400 (PEG_{3400}), polyethylene glycol 8000 (PEG_{8000}), and the like. When present as cosolvents in the subject formulations, the diols will usually range from about 5 to 60 %, usually from 5 to 55 % (w/v) of the formulation. The diols, especially higher molecular weight polyethylene glycols (i.e., such as polyethylene glycols having molecular weights of 1000 or higher), may function to adsorb water molecules which may be present in the formulations, thereby significantly reducing the possibility for precipitation of the cyclosporin from the formulation due to free water molecules.

In the subject formulations, the cosolvents themselves may impart desirable physical properties to the formulation, such as viscosity, stability and the like. Where desired, the formulation may further comprise additional agents which impart desired physical properties to the formulation, such as thickening agents, suspending agents, solidifying agents, and the like, where such agents include acacia, carboxymethyl-cellulose, hydroxypropylcellulose, lecithin, methyl cellulose, high molecular weight polyethylene glycols, e.g. those polyethylene glycols with molecular weights ranging from about 1000 to 10,000, usually 1000 to

8000 daltons, more usually 1000 to 6000, povidone, sodium alginate, tragacenth, and the like. Also present in the subject formulations may be a number of minor components which provide various functions, such as enzyme inhibitors, preservatives, antioxidants, antimicrobial agents, stabilizers, flavoring agents, and the like. The total amount of these thickening agents and other additives, when present in the formulation, will usually not be greater than 55 weight %, usually 45 weight %, more usually 25 weight % of the formulation. A number of excipients may also be present in the subject formulations, as is known in the art. Such additional agents are preferably anhydrous.

The formulations are substantially anhydrous meaning that they contain less than 1.0% vol/vol water, more preferably less than 0.1% vol/vol water.

The subject formulations are stable over a wide range of temperatures, where by stable is meant that the physical integrity of the formulation is not comprised, e.g. crystallization of the cyclosporin active agent does not occur. Included within the temperature range over which the subject formulations are stable are lower temperatures, such as those employed in refrigerated storage, where such lower temperatures typically range from about 0 to 15°C, more typically from about 2 to 8°C.

The subject formulations are suitable for administration in both liquid and in capsule form, *e.g.* hard and soft capsules. Methods of producing hard capsules comprising liquid formulations are known in the art and described in U.S. Patent Nos. 4,822,618 and 4,576,284, the disclosures of which are herein incorporated by reference. Generally, hard capsules that find use with the subject formulations will comprise two parts: a shell component and a cap component. The shell and cap components fit together to produce an enclosed cavity of defined volume sealed in a hard capsule shell. The shell and cap components may be fabricated from a hydrophilic polymer, such as starch or gelatin. In preparing the hard capsules, the liquid formulation will be poured into the shell component and then the capsule will be sealed by fitting the cap component over the shell component. The seal between the two

components may be secured, thereby preventing leakage of the enclosed formulation from the capsule, by using a sealant as described in EP 116744, the disclosure of which is herein incorporated by reference. To avoid degradation in the stomach, capsules comprising the subject formulations may be coated with an enteric coating which inhibits degradation of the capsule in the acidic environment of the stomach. A variety of enteric coatings are known in the art. *See for example*, U.S. Pat. No. 5,206, 219, the disclosure of which is herein incorporated by reference.

The subject formulations may be administered to subjects in need thereof in conjunction with one or more immunosuppressive agents, wherein other immunosuppressive agents which find use for co-administration with the cyclosporin formulations of the present invention include, for example, rapamycin, FK-506, mycophenolic acid, analogs and derivatives thereof, azathioprine, methotrexate, cyclophosphamide, corticosteroids, and any other immunosuppressive compound or molecule or analog thereof.

The subject formulations find use in immunosuppressive therapy. Immunosuppressive therapy is indicated in a wide variety of diseases, including idiopathic nephrotic syndrome, type I insulin-dependent diabetes, Behçet's syndrome, active Crohn's disease, aplastic anemia, severe corticosteroid-dependent asthma, psoriasis, rheumatoid arthritis, and other diseases where the immune system may play a pathogenic role. Of particular interest is the use of the subject formulations in transplant situations, including both allogeneic and xenogeneic organ, tissue or cell transplantation, where immunosuppression is desired to ensure maintained viability of the transplanted organ or tissue or cell following transplantation, *i.e.* to prevent graft rejection or prevent graft vs. host disease, *e.g.* following bone marrow transplantation.

In using the subject formulations to provide immunosuppressive therapy to a host, an effective amount of cyclosporin will be orally administered to achieve the desired level of immunosuppression in the host, depending on the particular condition to be treated. With

transplantation, usually an initial dosage of cyclosporin will be administered prior to operation or following surgery. Following transplantation of the donor organ to the host, the cyclosporin will generally be administered repeatedly, *i.e.* chronically, to the host to maintain immunosuppression. The initial dosage will be administered 4 to 12 hours prior to transplantation and may range from 5 to 18 mg/kg host, usually 5 to 15 mg/kg host. Following the operation, the initial dosage will usually be continued on a daily basis for a period of 1 to 3 weeks, usually 1 to 2 weeks. The dosage may then be tapered to a maintenance dosage of 3 to 10 mg/kg per day, usually 3 to 6 mg/kg per day. The rate at which the dosage is tapered to the maintenance level may range from 3 to 8 % per week and will usually be about 5 % per week. The dosage will typically be adjusted based on trough blood levels to maintain a concentration of 100 to 350 ng/ml, as measured by HPLC, RIA, ELISA or TDx assay. The subject formulations may be administered in conjunction with additional agents, where adjunct therapy is recommended and is known in the art. For example, the subject formulations may be administered in conjunction with adrenal corticosteroids, azathioprine, and the like.

Administration of the subject formulations in conjunction with transplantation of a donor organ to a host will result in a prolongation of the viability of the donor organ in the host as a result of suppression of the host's immune response to the presence of the donor organ. By "prolongation of viability" is meant that the donor organ remains viable in the host for a longer period of time than it would have had immunosuppressive therapy not been employed in conjunction with the transplantation. Thus, prolongation of viability includes maintenance of viability for an indefinite period of time. A donor organ is considered viable as long as it maintains functionality in the host environment.

The subject formulations may also be prepared as aqueous colloidal dispersions of cyclosporin nanoparticles having good bioavailability. In such cases, the nanoparticles are substantially spheric, the cyclosporin is present in an amorphous form, and the average size will generally be less

than about 1000 nm, greater than about 50 nm, generally in the range of about 200 - 800 nm, usually in the range of about 200-600 nm. Generally, at least about 50 weight percent of the total weight of cyclosporin will be present as particles in the indicated size range. Larger particles may be present, particularly as aggregates of nanoparticles, where the average diameter will usually be less than about 50 μm, more usually less than about 25 μm, the aggregates usually not exceeding 40 weight % of the total cyclosporin.

The amount of cyclosporin amorphous particles in the composition will be sufficient for therapeutic effect. Since the formulation may be formed by introduction into an aqueous medium prior to administration or directly into the gastric juices, the particular concentration cannot be stated, since the dilution in the stomach is uncertain. For preparation in an aqueous medium prior to oral administration, generally, the cyclosporin will be present at a weight percent of about 0.01-2.5, more usually from about 0.01-0.5 weight percent. The temperature of mixing may be in the range of about 10 to 50°C, usually in the range of about 20 to 40°C. Usually the mixing will involve stirring for sufficient time to provide the solution of the cyclosporin.

The colloidal amorphous suspension of the nanoparticles is sufficiently stable to allow for some standing prior to administration, frequently up to about 6 hours, more frequently up to about 3 hours.

Further details regarding the preparation and use of the nanoparticle cyclosporin formulations of the present invention may be found in PCT/US97/04627.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Several oral cyclosporin formulations according to the subject invention were prepared. The bioavailability of cyclosporin in many of the prepared formulations was then observed in rats and humans.

I. *Oral Cyclosporin Formulations*

The following oral Cyclosporin A formulations were prepared. In each case, the indicated amount of Cyclosporin A, surfactant and ethanol or propylene glycol were added to a 1.0 ml volumetric flask, and the final volume of 1.0 ml was optionally achieved by addition of a suitable volume of fatty acid ester and/or diol.

Formulation	Composition		
19	CsA EtOH Tween 80 IM	100 mg 0.1 ml 300 mg q.s. to 1.0 ml	(10% w/v) (10%) (0.278 ml) ((0.622 ml) (531 mg)
20	CsA EtOH Brij 30 IM	100 mg 0.05 ml 350 mg q.s. to 1.0 ml	(10% w/v) (5%) (0.368 ml) ((0.582 ml)(496 mg)
21	CsA PG Brij 30 IM	100 mg 0.05 ml 350 mg q.s. to 1.0 ml	(10% w/v) (5%) (0.368 ml) ((0.582 ml)(496 mg)
22	CsA EtOH Tween 80 EO	100 mg 0.1 ml 300 mg q.s. to 1.0 ml	(10% w/v) (10%) (0.278 ml) ((0.622 ml)(541 mg)
23	CsA EtOH Brij 30 EO	100 mg 0.05 ml 350 mg q.s. to 1.0 ml	(10% w/v) (5%) (0.368 ml) ((0.582 ml) (506mg)
24	CsA PG Brij 30 EO	100 mg 0.05 ml 350 mg q.s. to 1.0 ml	(10% w/v) (5%) (0.368 ml) ((0.582 ml) (506mg)
33	CsA EtOH Brij 30 IM	100 mg 0.1 ml 150 mg q.s. to 1.0 ml	(10% w/v) (10%) (0.158 ml) ((0.742 ml)(633 mg)
34	CsA EtOH Brij 30 EO	100 mg 0.1 ml 150 mg q.s. to 1.0 ml	(10% w/v) (10%) (0.158 ml) ((0.742 ml)(646 mg)
35	CsA EtOH Tween 80 PG	100 mg 0.105 ml 500 mg q.s. to 1.0 ml	(10% w/v) (10.5%) (0.463 ml) ((0.437 ml)(453 mg)

Formulation	Composition		
36	CsA EtOH Tween 80 PG EO	100 mg 0.1 ml 300 mg 100 mg q.s. to 1.0 ml	(10% w/v) (10 %) (0.278 ml) (0.097 ml) ((0.525 ml)(465 mg)
37	CsA EtOH Tween 80 PEG 400 EO	100 mg 0.1 ml 300 mg 100 mg q.s. to 1.0 ml	(10% w/v) (10 %) (0.278 ml) (0.088 ml) ((0.534 ml)(464 mg)
38	CsA EtOH Brij 30 PG EO	100 mg 0.1 ml 300 mg 100 mg q.s. to 1.0 ml	(10% w/v) (10 %) (0.316 ml) (0.097 ml) ((0.487 ml)(424 mg)
39	CsA EtOH Brij 30 PG EO	100 mg 0.1 ml 300 mg 200 mg q.s. to 1.0 ml	(10% w/v) (10 %) (0.316 ml) (0.193 ml) ((0.391ml)(340mg)
40	CsA PG Brij 30 EO	100 mg 300 mg 300 mg q.s. to 1.0 ml	(10% w/v) (290 ml) (0.316 ml) ((0.394 ml)(343 mg)
41	CsA EtOH Brij 30 Tween 80 EO	100 mg 0.05 ml 150 mg 100 mg q.s. to 1.0. ml	(10% w/v) (5%) (0.158 ml) (0.093 ml) ((0.649 ml) (565 mg)
42	CsA PG Brij 30 Tween 80 EO	100 mg 0.05 ml 150 mg 100 mg q.s. to 1.0. ml	(10% w/v) (5%) (0.158 ml) (0.093 ml) ((0.649 ml) (565 mg)
43	CsA EtOH Tween 80 PG	100 mg 0.10 ml 400 mg q.s. to 1.0 ml	(10% w/v) (10 %) (0.371 ml) (0.529 ml)
44 - liquid	CsA EtOH Tween 80 PEG_{400}	100 mg 0.125 ml 400 mg q.s. to 100 ml	(10% w/v) (12.5%) (0.371 ml) ((0.529 ml)(601 mg)
44 - hard capsule	CsA EtOH Tween 80 PEG_{400} PEG_{1000}	100 mg 0.125 ml 400 mg 0.25 ml 0.25 ml	(10% w/v) (12.5%) (0.371 ml)

Formulation	Composition		
45	CsA	100 mg	(10% w/v)
	EtOH	0.10 ml	(10%)
	Tween 80	300 mg	(0.278 ml)
	PG	approx.250mg	(0.243 ml)
	PEG_{400}	approx.250mg	(0.220 ml)
46	CsA	100 mg	(10% w/v)
	EtOH	0.10 ml	(10%)
	Tween 80	100 mg	(0.093 ml)
	PG	q.s. to 1.0 ml	(0.807 ml)
48	CsA	100 mg	(10% w/v)
	EtOH	0.10 ml	(10%)
	Tween 80	200 mg	(0.186 ml)
	PG	approx.250mg	(0.243 ml)
	PEG_{400}	approx.250mg	(0.220 ml)
49	CsA	100 mg	(10% w/v)
	EtOH	0.10 ml	(10 %)
	Tween 80	600 mg	(0.558 ml)
	PG	q.s. to 1 ml	(0.342 ml)
50	CsA	100 mg	(10% w/v)
	EtOH	0.10 ml	(10%)
	Tween 80	300 mg	(0.278 ml)
	PG	q.s. to 1.0 ml	(0.622 ml)
51	CsA	100 mg	(10% w/v)
	EtOH	0.10 ml	(10 %)
	Tween 80	200 mg	(0.186 ml)
	PG	q.s. to 1.0 ml	(0.714 ml)
52	CsA	100 mg	(10% w/v)
	EtOH	0.05 ml	(5%)
	Tween 80	400 mg	(0.371 ml)
	PG	q.s. to 1.0 ml	(0.579 ml)
100	CsA	125 mg	(12.5% w/v)
	EtOH	0.125 ml	(12.5%)
	Tween 80	200 mg	(0.186 ml)
	PEG_{300}	approx. 0.44 mg	
	PEG_{8000}	approx. 0.11 mg	
101	CsA	125 mg	(12.5% w/v)
	EtOH	0.125 ml	(12.5%)
	Tween 80	100 mg	(0.093 ml)
	PEG_{300}	approx. 0.52 mg	
	PEG_{8000}	approx. 0.13 mg	
102	CsA	125 mg	(12.5% w/v)
	EtOH	0.125 ml	(12.5%)
	Tween 80	100 mg	(0.093 ml)
	PEG_{300}	approx. 0.585 mg	
	PEG_{8000}	approx. 0.065 mg	
103	CsA	125 mg	(12.5% w/v)
	EtOH	0.125 ml	(12.5%)
	Tween 80	400 mg	(0.372 ml)
	PEG_{300}	approx. 0.28 mg	
	PEG_{3400}	approx. 0.07 mg	

Formulation	Composition		
104	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	125 mg 0.15 ml 100 mg approx. 0.496 mg approx. 0.124 mg	(12.5% w/v) (15%) (0.093 ml)
105	CsA EtOH Tween 80 PEG_{300} PEG_{3400}	125 mg 0.15 ml 400 mg approx. 0.256 mg approx. 0.064 mg	(12.5% w/v) (15%) (0.372 ml)
106	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	125 mg 0.125 ml 400 mg approx. 0.329 mg approx. 0.021 mg	(12.5% w/v) (12.5%) (0.372 ml)
107	CsA EtOH Tween 80	112 mg 0.158 ml 800 mg	(11.2 % w/v) (15.8%) (0.744 ml)
108	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	112 mg 0.158 ml 700 mg approx. 0.105 mg approx. 0.045 mg	(11.2 % w/v) (15.8%) (0.651 ml)
109	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	112 mg 0.158 ml 700 mg approx. 0.075 mg approx. 0.075 mg	(11.2 % w/v) (15.8%) (0.651 ml)
110	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	112 mg 0.158 ml 600 mg approx. 0.20 mg approx. 0.05 mg	(11.2 % w/v) (15.8%) (0.558 ml)
111	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	112 mg 0.158 ml 600 mg approx. 0.15 mg approx. 0.10 mg	(11.2 % w/v) (15.8%) (0.558 ml)
112	CsA EtOH Tween 80 PEG_{300} PEG_{8000}	112 mg 0.158 ml 500 mg approx. 0.315 mg approx. 0.035 mg	(11.2 % w/v) (15.8%) (0.465 ml)
113	CsA EtOH Tween 80 PEG_{300} PEG_{3400}	112 mg 0.158 ml 500 mg approx. 0.245 mg approx. 0.105 mg	(11.2 % w/v) (15.8%) (0.465 ml)

Formulation	Composition		
114	CsA	125 mg	(12.5 % w/v)
	EtOH	0.158 ml	(15.8%)
	Tween 80	300 mg	(0.279 ml)
	PEG_{300}	approx. 0.385 mg	
	PEG_{8000}	approx. 0.165 mg	
115	CsA	125 mg	(12.5 % w/v)
	EtOH	0.158 ml	(15.8%)
	Tween 80	300 mg	(0.279 ml)
	PEG_{300}	approx. 0.33 mg	
	PEG_{8000}	approx. 0.22 mg	
116	CsA	125 mg	(12.5 % w/v)
	EtOH	0.158 ml	(15.8%)
	Tween 80	400 mg	(0.372 ml)
	PEG_{300}	approx. 0.225 mg	
	PEG_{8000}	approx. 0.225 mg	

PG = Propylene Glycol; EtOH = ethanol; Brij 30 = polyoxyethylene (4) lauryl ether; Tween 80 = polyoxyethylene (20) mono sorbitan mono-oleate; IM = isopropyl myristate; EO = ethyl oleate

II. *Bioavailability Studies for Formulations 19-24 and 33-42*

The bioavailability of cyclosporin in formulations 19-24 and 33-42 was studied as follows. As a measure of bioavailability, the following pharmacokinetic parameters were determined: (a) the peak blood concentration of cyclosporin (C_{max}); (b) time required to attain C_{max} (T_{max}); and the area under the blood concentration time-curve time (AUC). In addition to formulations 19-24 and 33-42, the bioavailability of cyclosporin in SANDIMMUNE® Oral Solution (SO) under analogous conditions was observed for comparison purposes. For each of the above formulations, CsA-naive Sprague Dawley rats weighing 250-350 gm were fed pelletized standard food (Agway® 3000, Granville Mill, Greensboro, NC) and water *ad libitum*. One day prior to the experiment, silicone rubber cannulae were inserted into the right jugular and right femoral veins under light ether anesthesia. After overnight fast, CsA was administered by gavage.

Following administration, 200µl blood samples were collected from the jugular vein in 0.5 ml polypropylene microfuge tubes containing 0.3 mg of lyophilized Na EDTA and vortexed immediately for 10 sec. The sampling times for animals subjected to oral formulations were 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 hr after administration.

CsA, including some of its metabolites, was determined in whole blood by fluorescent polarization immunoassay (FPI)(TDx, Abbot Lab). Briefly, 150 μl of the whole blood sample were quantitatively transferred to a 1.5 ml microfuge tube. Cells were lysed and dissolved with 50 μl of a surfactant-containing solubilizing reagent. Proteins were then precipitated out with 300 μl of acetonitrile. After centrifugation, the supernatant was subjected to the FPI assay in a TDx Autoanalyzer following the procedure recommended by Abbott Diagnostics. Since the TDx assay was originally developed for human blood, some of the recommended procedures were modified as follows. A series of standard solutions of known CsA concentration were prepared by adding a known amount of CsA to rat blood treated with EDTA. When the CsA concentration in a sample was expected to be greater than 1.0 μg/ml, the blood sample was diluted 10-fold in a 0.1 M-phosphate buffer at pH 7.0. For diluted samples, another calibration curve was made using a series of standard solutions containing known amounts of CsA, which is volume-wise 10% in rat blood and 90% phosphate buffer.

Descriptive pharmacokinetic parameters were obtained from non-compartmental analyses. The peak concentration (C_{max}) and the time at which the peak concentration occurred (T_{max}) were estimated by inspection of the raw concentration-time profile for each rat. The area under the blood concentration-time curve (AUC) from time 0 through the last data point ($AUC_{0\rightarrow t}$) was calculated according to the linear trapezoidal procedure. The residual area under the tail of the blood concentration-time curve ($AUC_{t\rightarrow\infty}$) was estimated as the ratio of the final observed concentration (C*) to the first-order rate constant associated with the terminal elimination phase of the concentration-time profile (λ_z). The rate contact λ_z was determined by log-linear regression of the concentration-time data in the apparent terminal log-linear phase of the concentration-time profile (i.e., the final 3 to 5 data points, depending on the profile under analysis). The total AUC ($AUC_{t\rightarrow\infty}$) was taken as the sum of $AUC_{0\rightarrow t}$ and $AUC_{t\rightarrow\infty}$

The results for each formulation were compared with the results obtained for SO, and are provided in Figs. 1-3. The results demonstrate that, for the majority of the formulations, greater bioavailability of cyclosporin is achieved with the subject formulations as compared with SANDIMMUNE® Oral Solution (SO), as indicated by the higher AUC values of the subject formulations.

III. *Human Bioavailability of Formulations 35, 43-46 and 48-52.*

48 healthy males between the ages of 19 and 55 with no more than 20 % deviation from ideal weight were used as test subjects. A single dose, fasted, randomized, double-blinded, three-way crossover study was conducted. The 48 subjects were randomized into 6 groups of 8 subjects. Each group received a single 300 mg dose of cyclosporin from the above formulations, or SANDIMMUNE® Oral Solution (SO), on three different occasions, where each occasion was separated by a 7-day washout period.

Subjects were required to fast 10 hours prior to, and 4 hours after, dosing. Water was allowed ad lib during the study, except for a 1 hour period prior through 2 hours following dosing. Prior to dosing, a 15 ml blood sample was drawn. For administrations, 3 ml aliquots of formulation (300 mg) was combined with 200 ml of diluent and orally ingested. 10 ml blood samples were drawn at t= 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours. A post study 15 ml blood sample was also drawn.

Concentrations of cyclosporin A in the whole blood samples were assayed using the TDx (Abbott Diagnostics, N. Chicago, IL) according to the manufacturer's instructions.

Non-compartmental pharmacokinetics were derived using standard methods. The maximum whole blood concentration (C_{max}) and the time of its occurrence (T_{max}) were compiled from the concentration-time data. The area under the blood concentration time curve (AUC) was calculated by the linear trapezoidal rule to the last blood concentration above the limit of sensitivity (25 ng/ml) and extrapolated to infinity.

The observed C_{max}, T_{max} and AUC values for each formulation were averaged. The average values for each formulation are provided in Figures 4-6. The results demonstrate that for each formulation tested, Cmax occurred at least twice as fast as with SANDIMMUNE® Oral Solution (SO) under the same conditions. Furthermore, the AUC observed for the test formulations was at least 2000 ng•hr/ml greater than that observed for SANDIMMUNE® Oral Solution (SO) under the same conditions. Based on these results, formulations 35, 43-46 and 48-52 provide for greater bioavailability than SANDIMMUNE® Oral Solution (SO).

IV. *Cold Temperature Characteristics of Liquid Formulations 35 and 44*

Cyclosporin A liquid formulations 35, 44 and the NEORAL® oral solution were stored at various low temperatures and their physical characteristics determined. Specifically, liquid cyclosporin A formulations 35, 44 and the NEORAL® oral solution were stored overnight either at -10° C or from 2 to 8° C. At -10° C, both formulation 44 and the NEORAL® oral solution solidified, whereas formulation 35 did not, although it became quite viscous. Interestingly, the cold formulation 35 solution did not show any cyclosporin A crystalline precipitate. When each of the formulations were taken out into ambient temperature, the solidified formulation 44 thawed more rapidly than the solidified NEORAL® oral solution.

When stored at from 2 to 8° C, the NEORAL® oral solution solidified into a paste whereas both formulations 35 and 44 remained as a viscous liquid solution. Neither cold solution of formulation 35 or 44 showed any cyclosporin A precipitate.

From the above results and discussion, it is evident that novel cyclosporin formulations having high bioavailability are provided. The subject formulations are capable of comprising high concentrations of cyclosporin and are storage stable over a wide range of temperatures, including low temperatures commonly used in refrigeration. The subject formulations are amenable to delivery in capsule form, including hard capsule form, providing for ease of storage and handling.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

* C L M S *

WHAT IS CLAIMED IS:

1. An oral cyclosporin formulation consisting essentially of: cyclosporin;

at least one alkanol solvent of from 2 to 3 carbon atoms;

at least one non-ionic polyoxyalkylene surfactant, wherein said surfactant is selected from the group consisting of polyoxyethylene alcohols and fatty acid monoesters of ethoxylated polyols of from 4 to 6 carbon atoms; and

at least one polyglycol, wherein at least one of said at least one polyglycols has a molecular weight in the range from 800 to 10,000 daltons.

2. The formulation of Claim 1 wherein said polyglycol comprises polyethylene glycol.

3. The formulation according to Claim 2, wherein said alkanol solvent is from about 5 to 75 % (v/v) of said formulation.

4. The formulation according to Claim 2, wherein said alkanol solvent is from about 10 to 25 % (v/v) of said formulation.

5. The formulation according to Claim 4, wherein said alkanol solvent is absolute ethanol.

6. The formulation according to Claim 2, wherein said at least one non-ionic polyoxyalkylene surfactant is from about 5 to 80 % (w/v) of said formulation.

7. The formulation according to Claim 2, wherein said at least one non-ionic polyoxyalkylene surfactant is from about 10 to 50 % (w/v) of said formulation.

8. The formulation according to Claim 2, wherein said at least one non-ionic polyoxyalkylene surfactant is from about 20 to 75 % (v/v) of said formulation.

9. The formulation according to Claim 2, wherein said at least one non-ionic polyoxyalkylene surfactant is selected from the group consisting of polyoxyethylene (20) mono sorbitan mono-oleate and polyoxyethylene (4) lauryl ether.

10. The formulation according to Claim 2, wherein said at least one polyethylene glycol comprises a polyethylene glycol having a molecular weight in the range of from about 1000 to 8000 daltons.

11. The formulation according to Claim 2, wherein said at least one polyethylene glycol is a mixture of two different polyethylene glycols each having a different molecular weight.

12. The formulation according to Claim 2, wherein said at least one polyethylene glycol is from about 20 to 80 % (w/v) of said formulation.

13. The formulation according to Claim 1, wherein cyclosporin is present at a concentration of 100 mg/ml, absolute ethanol is present at a concentration of 12.5 % and polyoxyethylene (20) mono sorbitan mono-oleate is present at a concentration of 400 mg/ml.

14. The formulation according to Claim 1 which is present in a hard capsule.

15. An oral cyclosporin formulation consisting essentially of: cyclosporin;

at least one alkanol solvent of from 2 to 3 carbon atoms; and

at least one non-ionic polyoxyalkylene surfactant, wherein said surfactant is selected from the group consisting of polyoxyethylene alcohols and fatty acid monoesters of ethoxylated polyols of from 4 to 6 carbon atoms.

16. The formulation according to Claim 14, wherein said alkanol solvent is from about 5 to 75 % (v/v) of said formulation.

17. The formulation according to Claim 14, wherein said alkanol solvent is from about 10 to 25 % (v/v) of said formulation.

18. The formulation according to Claim 16, wherein said alkanol solvent is absolute ethanol.

19. The formulation according to Claim 14, wherein said at least one non-ionic polyoxyalkylene surfactant is from about 5 to 80 % (w/v) of said formulation.

20. The formulation according to Claim 14, wherein said at least one non-ionic polyoxyalkylene surfactant is from about 65 to 80 % (w/v) of said formulation.

21. The formulation according to Claim 14, which is present in a hard capsule.

22. An oral cyclosporin formulation comprising cyclosporin and a physiologically acceptable carrier wherein said formulation is substantially anhydrous.

This Sheet Has Been Replaced

23. A method for achieving immunosuppression in a host, said method comprising:

administering to said host an oral cyclosporin formulation according to any one of Claims 1-22, whereby immunosuppression in said host is achieved.