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(54) Title: OPTHALMIC COMPOSITIONS CONTAINING A CYCLOSPORIN (57) Abstract An ophthalmic composition particularly in the form of eye-drops suitable for the treatment of diseases of the eye and surrounding areas. The composition contains a cyclosporin and a surfactant selected from polyoxyethylene fatty acid esters, polyoxyethylene alkylphenyl ethers and polyoxyethylene alkyl ethers, or mixtures thereof.		

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OPHTHALMIC COMPOSITIONS CONTAINING A CYCLOSPORIN

This invention relates to ophthalmic compositions, particularly eye-drop formulations, comprising a cyclosporin as active ingredient and being suitable for the treatment of diseases of the eye and surrounding areas.

The cyclosporins comprise a large and recognized class of peptide compounds having pharmaceutical utility, for example immunosuppressant, anti-inflammatory, and/or anti-parasitic activity and/or activity in abrogating tumor resistance to anti-neoplastic or cytostatic drug therapy. The cyclosporins include, for example, naturally occurring fungal metabolites, such as the cyclosporin A, B, C, D and G, as well as a wide variety of synthetic and semi-synthetic cyclosporins, for example the dihydro- and iso-cyclosporins (see e.g. US Patents 4,108,985; 4,210,581 and 4,220,641), [(D)-Ser]⁸-Ciclosporin (see US Patent 4,384,996), [O-acetyl, (D)-Ser]⁸-Ciclosporin (see US Patent 4,764,503), [β -fluoro-(D)Ala]⁸-Ciclosporin (see UK Patent Application 2,206,119A), [Val]²-[(D)methylthio-Sar]³- and [Dihydro-MeBmt]¹-[Val]²-[(D)methylthio-Sar]³-Ciclosporin [see US Patent 4,703,033], [O-(2-hydroxyethyl)-(D)Ser]⁸-Ciclosporin, and [3'-deshydroxy-3'-keto-MeBmt]¹-[Val]²-Ciclosporin and many more.

Of the cyclosporins, the most widely investigated to date is cyclosporin A, being commercially available under the Registered Trade Mark SANDIMMUN or SANDIMMUNE. Cyclosporin A has been shown to suppress selectively a variety of T-lymphocyte functions, including prevention of maturation and expression of sensitized T-lymphocytes in cell mediated immune responses, and is now successfully and widely used in the suppression of organ transplant rejection. Cyclosporin A has also been used systemically in the treatment of intraocular inflammatory or autoimmune diseases, such as uveitis. However, because of the side effects associated with systemic therapy, Cyclosporin A has had only limited use in treating conditions of the eye.

Effective topical administration of Cyclosporin A to the eye would reduce or eliminate to a large extent the systemic side effects by restricting activity to the locus of the condition being treated and proposals to this effect have been made, (see for example US Patent 4,649,047). However, utility and effectiveness of Cyclosporin A in treating diseases and conditions of the eye has been hindered until now by the lack of suitable

eye-drops which are acceptable to the eye. Eye-drops are required which do not cause patient discomfort and which permit a convenient administration regimen and do not require unduly frequent administration, while providing adequate drug substance delivery both to the external and, in particular, the internal regions of the eye. A further difficulty is the very poor solubility of cyclosporin A in water. This leads often to precipitation of cyclosporin A from aqueous-based eye-drops causing strong irritation of the eye.

Efforts have been made to overcome these difficulties by dissolving cyclosporin A in vegetable oils (Ophthalmology, 96, 1144-1150 (1989)) and by clatherating cyclosporin A with cyclodextrin (Japanese unexamined Patent Publication SHO-64-85921/1989).

In oily solution, however, cyclosporin A is poorly distributed in the eyes (Folia Ophthalmologica Japonica, 40, (5), 902-908 (1989)), and a high concentration (2%) of cyclosporin A is needed for clinical treatment (Ophthalmology, 96, 1144-1150 (1989)). Further, these oily eye drops tend to cause a disagreeable feeling to the eyes.

Also, whereas cyclosporin eye-drops solubilized by clatherating with cyclodextrin certainly have an improved distribution in the eyes (Folia Ophthalmologica Japonica, 40, (5), 902-908 (1989)), the dissolved cyclosporin A is however again precipitated. As indicated by experiments in rabbits, this provokes strong irritation in at least the anterior part of eyes and induces redness and edema of conjunctival palpebrae and secretion from the conjunctiva, causing problems for application of cyclosporin A in this form.

There is thus an urgent need to develop a topical, ophthalmic formulation which causes less irritation to the eyes, has a better distribution of cyclosporin A in the eyes and has no problems of precipitation of cyclosporin.

In an attempt to solve these problems, studies have been conducted with various surfactants which are currently used for formulating medical substances with low solubility in water, especially the most commonly used surfactants polysorbate 80 and polyoxyethylene hydrogenated castor oil. However, polysorbate 80 was found to have a poor solubilizing effect, when used for the preparation of eye-drops, and the dissolution of cyclosporin was not sufficient. Polyoxyethylene hydrogenated castor oil was found to strongly irritate the eyes when used in eye-drops.

Thus these commonly used surfactants proved not to be successful for the preparation of eye-drops which contain a high concentration of cyclosporin and have

decreased irritation to the eyes.

Surprisingly these difficulties may be overcome by formulating the cyclosporin in a surfactant selected from polyoxyethylene fatty acid esters, polyoxyethylene alkylphenyl ethers, and polyoxyethylene alkyl ethers, or mixtures thereof.

5 Accordingly in one aspect this invention provides an ophthalmic composition comprising a cyclosporin, especially cyclosporin A, and a surfactant selected from polyoxyethylene fatty acid esters, polyoxyethylene alkylphenyl ethers, and polyoxyethylene alkyl ethers, or mixtures thereof.

The ophthalmic compositions are preferably formulated as eye-drop formulations.
10 Preferably they are aqueous based.

With these ophthalmic compositions, very good therapeutic results may be obtained even when the cyclosporin is present in low concentrations; for example within the range of from about 0.005 to about 1.0% (w/v), preferably about 0.005 to about 0.1% (w/v), and, more desirably from about 0.01 to about 0.075 % (w/v). As used in this specification, 1%
15 (w/v) is equivalent to 1 g per 100 ml.

Preferred polyoxyethylene fatty acid esters are based on saturated fatty acids, preferably not containing any substituent. The chain length may be from 14 to 22 carbon atoms, preferably 16 to 18 carbon atoms. A preferred fatty acid is stearate. Preferably the ester is a mono ester. Preferably the polymerization number of the polyoxyethylene
20 moiety is from about 20 to about 60. A preferred example is polyoxyl 40 stearate. An example is the polyoxyl 40 stearate known under the brand name Myrj 52 (available from Atlas Chemie, Essen, Germany).

Preferred polyoxyethylene aralkyl esters are based on a phenol substituted by one or more alkyl groups; the alkyl groups for example having from 4 to 10 carbon atoms,
25 preferably 8 or 9 carbon atoms. Preferably the phenyl group has only one alkyl group as substituent. Preferably the polymerization number of the polyoxyethylene moiety is from about 1 to about 50, more preferably around 40. A preferred example has properties as laid down in the Cosmetic, Toiletry & Fragrance Association, Inc (CTFA) cosmetic ingredients directory. Examples are octoxynols, for example those known under the brand
30 name "Triton" and obtainable from Rohm and Haas, Philadelphia, USA.

Preferred polyoxyethylene alkyl ethers are based on fatty alcohols having, for

example, from 4 to 20 carbon atoms. Preferably the polymerization number of the polyoxyethylene moiety is from 10 to 60. An example is the polyoxyethylene alkyl ether known as Cetomacrogol 1000 which has an acid value of less than 0.5 a hydroxyl value of 40 to 52.5 and an refractive index of 1.448 to 1.452.

5 The preferred surfactant is a polyoxyethylene fatty acid ester and more preferably polyoxyl 40 stearate.

Preferably the weight ratio of surfactant to cyclosporin is from about 10:1 to about 50:1.

10 The concentration of surfactant in the ophthalmic compositions is desirably within the range of from about 0.1 % to about 3.0 % (w/v), and, more desirably from about 0.15 to about 2.5 % (w/v), and especially 0.75% to 2.5% (w/v).

15 The ophthalmic composition may also include thickening (viscosity-increasing or viscoelastic) agents since these agents are useful for improving the distribution of the cyclosporin in the eyes. Preferred thickening agents are cellulose and cellulose derivative thickening agents such as alkyl celluloses and hydroxyalkylcelluloses and also non-cellulose thickening agents such as carboxyvinyl polymers, polyvinyl polymers and polyvinylpyrrolidones. Suitable examples are methyl cellulose, and especially hydroxypropyl methylcellulose 8 for example Nos. 2208 or 2906 as defined in the Japanese and US Pharmacopeia), and hydroxyethyl cellulose. Suitable polyvinyl polymers are polyvinylacetates and polyvinylalcohols and suitable polyvinylpyrrolidones are poly-N-vinylpyrrolidones and vinylpyrrolidone co-polymers such as vinylpyrrolidone-vinylacetate co-polymers.

20 The concentration of the thickening agents to be used is not critical. Typically it is within the range of about 0.05 to about 5.0 % (w/v), and more desirably, from about 0.1 to about 3.0 % (w/v).

25 To ascertain the distribution of the cyclosporin in the eyes, eye-drops containing cyclosporin A and a thickening agent were applied to rabbits. An eye-drop preparation in which cyclosporin A was solubilized with cyclodextrin was used as the control. The test showed that the eye-drops containing the thickening agent attained 1.7 times higher cyclosporin A concentration in the cornea than that obtained with the control.

30 With the excellent distribution of cyclosporins in the eyes obtained by the

ophthalmic compositions, it is possible to obtain sufficient therapeutic efficacy of cyclosporins A in the eye-drops even at low concentrations of cyclosporins in the composition.

Preferably an anti-oxidant is present but this is not essential. The choice of anti-oxidants which may be used is not critical but it is desirable to use butylated hydroxyanisol or ascorbic acid, and preferably sodium thiosulfate or butylated hydroxytoluene. Also, instead of an anti-oxidant, the ophthalmic composition may be stored in a container containing nitrogen and optionally including a free oxygen absorber (for example, Fe).

Stability studies were conducted using butylated hydroxytoluene as a representative anti-oxidant. In the studies it was found that the amount of cyclosporin (especially cyclosporin A) present after storage for one month at 40°C without anti-oxidant was reduced down to 85.3%. On the other hand there was no significant loss of cyclosporin in the composition containing an anti-oxidant as defined above. This showed extremely high stability. For example the composition of Example 5D as described hereinafter showed no appreciable decomposition after 3 months at 25°C and 40°C. However not all compositions require an anti-oxidant for stability.

Naturally the anti-oxidant should be present at concentrations below which it causes irritation of the eyes. Typically the concentration of anti-oxidant should be within the range from about 0.00005 to about 0.1 % (w/v), more desirably within the range of from about 0.0001 to about 0.01 % (w/v) to avoid irritation of the eye.

The preferred ophthalmic composition comprises from about 0.01 to about 0.075 % (w/v) of cyclosporin A; from about 0.15 to about 2.5 % (w/v) of polyoxyl 40 stearate; from about 0.1 to about 3.0 % (w/v) of a cellulose thickening agent selected from hydroxypropyl methylcellulose or hydroxyethylcellulose; and from about 0.0001 to about 0.01 % (w/v) of an anti-oxidant selected from butylated hydroxytoluene or sodium thiosulfate.

The combination of the surfactants such as polyoxyl 40 stearate or polyoxyethylene cetyl ether or polyoxyethylene octylphenyl ether with a cyclosporin (especially cyclosporin A) yields an excellent eye-drop preparation causing less irritation to the eyes, providing better distribution in the eyes, having a low concentration of cyclosporin A minimizing the

problem of its precipitation, and yet having higher stability. Moreover the inclusion of an anti-oxidant and an cellulose thickening agent may further improve the distribution of the cyclosporin in the eyes and the stability.

5 If desired other excipients may be present, for example an isotonic agent, buffer, preservative, and/or pH-controlling agent. Sterile purified water in appropriate amounts may be present to obtain the desired eye-drop preparation.

Conveniently ethanol is present, for example at a concentration of about 0.01 to 0.5% (w/v).

10 The pH of the ophthalmic composition may be within the range which is normally used for ophthalmic preparations, but is desirably within the range of 4 to 8.

The ophthalmic compositions may be prepared by mixing the cyclosporin with the above-mentioned surfactant, and as desired mixing in the thickening agent and the anti-oxidant, and other excipients.

15 The ophthalmic compositions may be filled in appropriate containers to facilitate administration to the eye, for example a dropper fitted with a suitable pipette.

Accordingly, in another aspect this invention provides an ophthalmic composition as defined above in a container appropriate for ophthalmic application of the composition, for example appropriate for application of the ophthalmic composition to or at the surface of the eye (for example to the cornea or corneal epithelium).

20 The invention also provides a method of treating a disease or condition of the eye or of the surrounding or associated organs or tissues, in a subject in need thereof, in particular of treating immune mediated or inflammatory diseases organs or tissues, which method comprises administering an ophthalmic composition as defined above topically to the eye, for example to or at the surface of the eye (for example to the cornea or corneal epithelium). The particular sections, segments or tissues of the eye to which the method is applicable are as described in this specification. Particular diseases or conditions of the eye to which the method is applicable are similarly as described in this specification.

In a further aspect the invention provides an ophthalmic composition as defined above for use in a method as defined above.

30 In another aspect this invention provides the use of a cyclosporin in the preparation of an ophthalmic composition for use in a method as defined above.

The following examples are illustrative of ophthalmic compositions of the invention. Further details of components are given in "Lexicon for Pharmazie, Kosmetik and angrenzende Gebiete" by H. P. Fiedler, 3rd Edition, 1989 published by Editio Cantor Aulendorf, Germany; Handbook of Pharmaceutical Excipients, 1st Edition, 1986, joint
 5 publication of American Pharmaceutical Association, Washington, USA and the Pharmaceutical Society of Great Britain, London, England; or from the relevant manufacturers. In the following examples, viscosity is measured as Newton viscosity at 25°C, for example measured in a viscosity measuring apparatus type CV 20 produced by HAAKE.

10 The polyoxyl 40 stearate used in the Examples is KIKKOL (Registered Trade Mark) MYS-40, a product of Nikko Chemicals Co. Ltd. Japan. It has a congealing point of 39 to 44°C, an acid value of not more than 1 and a saponification value of 25 to 35.

The polyoxyethylene octyl phenyl ether used in the Examples is NONION HS 240, a product of Nippon Oil and Fats Co. Ltd., Japan. The mean polymerization number is 40
 15 and the mean molecular weight is 1968.

The hydroxy propyl methylcellulose (also referred to as HPMC) used in the Examples is METOLOSE 65SH-4000 a product of Shin-Etsu Chemical Co. Ltd. Japan, corresponding to hydroxypropyl methyl cellulose 2906.

20 EXAMPLE 1: Eye-drop formulation (in 100 ml)

	Cyclosporin A	0.05 g
	Polyoxyl 40 stearate	2.0 g
	Hydroxypropyl methylcellulose	0.3 g
25	Butylated hydroxytoluene	0.001 g
	Ethanol	0.1 g
	Sodium chloride	0.73 g
	Sodium dihydrogen phosphate	0.2 g
	Sodium edethate	0.1 g
30	Sodium hydroxide	q.s.
	Sterile purified water	q.s.

Method for preparation:

The cyclosporin A is dissolved in an ethanolic solution containing the butylated hydroxytoluene, and the polyoxyl 40 stearate is added to it. After warming the mixture, the hydroxypropyl methylcellulose (which is dissolved in advance into a small amount of sterilized purified water) is added and the remaining sterile purified water is added to the mixture to yield a clear solution. Lastly, the sodium chloride, sodium dihydrogen phosphate and sodium edethate is added to the mixture, and the pH is adjusted to 6.0 using sodium hydroxide solution.

The following formulations can be prepared in the same manner as mentioned above.

EXAMPLE 2: Eye-drop formulation (in 100 ml)

	Cyclosporin A	0.025 g
15	Polyoxyl 40 stearate	0.5 g
	Hydroxypropyl methylcellulose	0.2 g
	Butylated hydroxytoluene	0.0005 g
	Ethanol	0.1 g
	Sodium chloride	0.73 g
20	Sodium dihydrogen phosphate	0.2 g
	Sodium edethate	0.1 g
	Sodium hydroxide	q.s.
	Sterile purified water	q.s.

EXAMPLE 3: Eye-drop formulation (in 100 ml)

	Cyclosporin A	0.075 g
	Polyoxyl 40 stearate	2.5 g
5	Hydroxypropyl methylcellulose	0.2 g
	Butylated hydroxytoluene	0.002 g
	Ethanol	0.1 g
	Sodium chloride	0.73 g
	Sodium dihydrogen phosphate	0.2 g
10	Sodium edethate	0.1 g
	Sodium hydroxide	q.s.
	Sterile purified water	q.s.

EXAMPLE 4: Eye-drop formulation (in 100 ml)

15	Cyclosporin A	0.1 g
	Polyoxyl 40 stearate	3.0 g
	Hydroxyethyl cellulose	0.3 g
	Sodium thiosulfate	0.01 g
20	Ethanol	0.1 g
	Sodium chloride	0.73 g
	Sodium dihydrogen phosphate	0.2 g
	Sodium edethate	0.1 g
	Sodium hydroxide	q.s.
25	Sterile purified water	q.s.

EXAMPLE 5:

	A	B	C*	D	
	Cyclosporin A	0.075%	0.075%	0.05%	0.05%
	Polyoxyl 40 stearate	1.125%	1.125%	0.75%	1 %
5	HPMC	-	-	0.3%	0.3&
	Butylated hydroxy- toluene	-	-	-	0.0001%
	Propylene glycol	-	0.25%	-	-
	Ethanol	0.1 %	-	0.1%	0.1%
10	Concentrated glycerin	2.1%	1.92%	-	-
	NaH ₂ PO ₄ ·2H ₂ O	0.2%	0.2%	0.2%	0.2%
	Sodium edethate	-	-	0.1%	0.1
	NaCl	-	-	0.72%	0.72%
	HCl/NaOH (to pH 7)	q.s.	q.s.	-	-
15	Purified water	q.s.	q.s.	q.w.	q.w.

* Viscosity 6.2 cps

In the above examples the polyoxyl 40 stearate may be replaced by an equivalent
20 amount of polyethylene octyl phenyl ether or polyoxyethylene alkyl ether.

The ophthalmic compositions are useful for the same indications as other topical
ophthalmic compositions containing cyclosporins, for example diseases affecting the
cornea, the aqueous, the lens, the iris, the ciliary, the choroid or the retina. The
ophthalmic compositions are useful particularly for the treatment of an autoimmune or
25 inflammatory disease or condition of the eye or of the surrounding or associated organs or
tissues, that has undesirably elevated immuno-response or inflammatory reaction or event
as part of its etiology. The ophthalmic compositions are useful preferably for treating the
anterior or posterior segment of the eye. For example for the treatment of anterior or
posterior uveitis, chronic keratitis, keratoconjunctivitis sicca, vernal keratoconjunctivitis,
30 conjunctivitis, including vernal conjunctivitis, or in keratoplasty. The ophthalmic
compositions may also be used in the treatment of immunoreactive graft rejection post

corneal transplantation, Behçet disease, and autoimmune corneal diseases such as Mooren's ulcer, ocular pemphigus, and rheumatoid ulcer.

The utility of the ophthalmic compositions and advantageous therapeutic properties can be observed in standard animal models and in standard clinical tests; for example by
5 administering, a few times a day, 0.05 ml to 0.5 ml, preferably 0.1 ml to 0.2 ml, of an ophthalmic composition containing 0.005% to 1.0%, preferably 0.01% to 0.5%, (by weight) of cyclosporin to the eyes of patients exhibiting diseases or conditions of the eye as set forth above. The tolerance of and transfer of the cyclosporin into the eye may be shown in tests as described below.

10 The optimal dosage to be administered to a particular patient will vary from patient to patient and from disease to disease and must be considered carefully by the treating physician. However doses in the range of 0.05 ml to 0.5 ml, preferably 0.1 ml to 0.2 ml, of an ophthalmic composition containing 0.005% to 1.0%, preferably 0.01% to 0.5%, (by weight) of cyclosporin may be used. Satisfactory results are obtained by administering
15 droplets of about 0.05 ml a few times a day; for example 1 to 5 times a day.

The excellent tolerance of the ophthalmic compositions may be determined in conventional tests. For example the compositions are administered topically to groups of 6 rabbits. One drop (about 50 microlitre) at a time is instilled intra-ocularly into one eye, every 30 minutes, for 5 hours (ten administrations). The other eye (untreated) is used as a
20 control. The eye is then examined microscopically for irritation at 1, 5 and 18 hours after the final administration, and the effect of the composition judged with respect to: opacity and opaque area in the cornea; the iris, redness and edema of conjunctival palpebrae, redness of conjunctival bulbi, state of mictating membrane and secretion of conjunctiva.

The ophthalmic compositions show excellent tolerability. For example the
25 compositions of Examples 5A and B showed a zero score in all the above mentioned evaluations, except for the evaluation of redness of conjunctival palpebrae. In this evaluation, however, only a marginal redness of conjunctival palpebrae was noted at 1 hour after final administration and the results are very close or identical to results obtained with physiological saline (0.9% sodium chloride).

30 The transfer of Cyclosporin A into the cornea (without epithelium) from the ophthalmic compositions may be evaluated by conventional tests. In one test 50 microlitres

of test solution are installed into the eyes of groups of 5 rabbits and the amount of Cyclosporin A in the cornea measured. The animals are sacrificed by injection of sodium pentobarbital through the auditory vein 30 minutes after administration. The eye balls are enucleated, their surface cleaned with 1 ml of physiological saline and the corneal
5 epithelium removed with a fine razor. The cornea is then resected by cutting it along its circumference, and washing it 3 times with physiological saline and drying it completely free of water using a sheet of filter paper. The cornea is weighed, and kept at -80°C.

The cornea is then homogenized in 1 ml methanol while cooling with ice and the homogenate centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatant
10 is collected. 1 ml methanol are added, the mixture subjected to Boltex treatment and then centrifuged again. The supernatants are combined, evaporated at 40°C to 50°C and 1 ml methanol is added to the residue. 50 microlitres of the solution are measured out and the amount of Cyclosporin A therein determined by standard radioimmunoassay procedures.

The excellent transfer of Cyclosporin A into the cornea is for example shown by the
15 composition of Example 5C in the above test. A mean value of 479.2 ng/g (S.D. +/- 245.8) is observed.

For the purpose of such trials, or for practical therapeutic use, the ophthalmic compositions (for example the composition of example 1) are suitably administered at or to
20 the surface of the eye in individual amounts. For example of from about 0.1 to 0.2 ml from 1 to 4x daily, depending upon the particular disease or condition to be treated, its clinical status and the effect desired. Marked improvement in condition as compared with untreated controls are observable with continuance of treatment, for example over a period of 1 to 2 weeks and upwards. The ophthalmic compositions are found to be well tolerated by subjects undergoing such therapy, with no significant or untoward irritation.

CLAIMS:

1. An ophthalmic composition comprising a cyclosporin and a surfactant selected from polyoxyethylene fatty acid esters, polyoxyethylene alkylphenyl ethers and polyoxyethylene alkyl ethers, or mixtures thereof.
- 5
2. An ophthalmic composition according to claim 1 and further comprising a thickening agent.
- 10
3. An ophthalmic composition according to claim 2 in which the thickening agent is selected from cellulose thickening agents, carboxyvinyl polymers, polyvinyl polymers and polyvinylpyrrolidones.
4. An ophthalmic composition according to claim 1 comprising from about 0.01 to about 0.075 % (w/v) of cyclosporin A; (1) from about 0.15 to about 2.5 % (w/v) of polyoxyl 40 stearate; (2) from about 0.1 to about 3.0 % (w/v) of a cellulose thickening agent selected from hydroxypropyl methylcellulose or hydroxyethylcellulose; and (3) from about 0.0001 to about 0.01 % (w/v) of an anti-oxidant selected from butylated hydroxytoluene or sodium thiosulfate.
- 15
- 20
5. An ophthalmic composition according to any one of claims 1 to 4 further comprising an aqueous carrier.
6. An ophthalmic composition according to any one of claims 1 to 3 in which the cyclosporin is cyclosporin A.
- 25
7. An ophthalmic composition according to any one of claims 1 to 4 in a container appropriate for topical ophthalmic application of the composition.
- 30
8. A method of treating a disease or condition of the eye or of the surrounding or associated organs or tissues in a subject in need thereof, which method comprises

administering a composition according to any one of claims 1 to 4 topically to the eye.

9. An ophthalmic composition as claimed in any one of claims 1 to 4 for use in a method of treating a disease or condition of the eye or of the surrounding or associated organs or tissues in a subject in need thereof.
- 5
10. A cyclosporin for use in the preparation of a composition as claimed in any one of claims 1 to 4.

INTERNATIONAL SEARCH REPORT

PCT/EP 93/01123

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A61K9/00; A61K37/02		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	FR,A,2 638 089 (SANDOZ) 27 April 1990 see claims ---	1-10
Y	FR,A,2 636 534 (SANDOZ) 23 March 1990 see claims 1,7-9,12,14,16-22,28-30 see examples 2,5,6 ---	1-10
Y	GB,A,2 211 848 (SANDOZ) 12 July 1989 see claims 1,14-29 see page 14, line 4 - line 10 see page 14, line 26 - line 31 see page 16, paragraph d) see page 19, paragraph e) see example 4 ---	1-10
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<p>¹⁰ Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
09 AUGUST 1993	17. 08. 93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	SCARPONI U.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	US,A,4 649 047 (R.KASWAN) 10 March 1987 cited in the application see claims	1-10
A	EP,A,0 294 239 (SANKYO) 7 December 1988 cited in the application see claims see page 5,experiment 1 see page 6,experiment 2	1-10
P,Y	DATABASE WPIL Week 9315, Derwent Publications Ltd., London, GB; AN 93-121283 (15) & JP,A,05 058 906 (SANKYO) 9 March 1993 see abstract	1-10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 93/01123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claim 8 is directed to a method of treatment of the human body by therapy (rule 39.1(4)PCT), the search has been carried out and based on the alleged effects of the composition.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

EP 9301123
SA 74009

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

09/08/93

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