Cyclosporine A Formulation Affects Its Ocular Distribution in Rabbits

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INTRODUCTION

Systemic Cyclosporine A (CsA) administration averts graft rejection after organ transplantation. In the eye, CsA is also beneficial in the treatment of autoimmune diseases, uveitis, Bechet's disease, Sjögren's syndrome, keratoconjunctivis sicca, and corneal transplantation (1). It has been suggested that topical rather than systemic CsA application could also be therapeutic, without causing systemic side effects, in the treatment of ocular disease. This may be possible because much less CsA can penetrate into the bloodstream after its topical instillation in either an aqueous- or oil-based medium. It is expected that penetration from an aqueous medium will be even less than in oil because its solubility in water is much less. Various attempts were made to develop ophthalmic formulations that improve ocular CsA penetration, including an alpha-cyclodextrin vehicle (2), vegetable oils (3), liposomes (4), collagen shields (5), micro- or nanospheres (6), and oil-in-water emulsion (7). However, none of these deliver therapeutic amounts of CsA into target ocular tissues with low ocular toxicity.

In this work, we compared in rabbit ocular tissues CsA pharmacokinetics and distribution resulting from its topical application as an oil-based medium, o/w emulsion, and CsA aqueous clear solution containing a surfactant. Our results suggest that only an aqueous solution containing the nonionic surfactant MYS-40 delivers therapeutic levels of CsA.

MATERIAL AND METHODS

Chemicals

CsA was kindly donated by Novartis Pharma (Tokyo, Japan). Polyoxyethylene 20 sorbitan monooleate (Tween 80) was purchased from Kao Corporation (Tokyo, Japan). Polyoxyl 40 stearate (MYS-40) and polyoxyl 60 hydrogenated castor oil (HCO-60) were purchased from Nikko Chemical Co., Ltd (Tokyo, Japan). Pemulen® TR-2 polymeric emulsifier was supplied by BF Goodrich (Cleveland, OH). Hydroxypropyl Methylcellulose (HPMC, 65SH4000 grade) was purchased from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Tritium labeled CsA (37 MBq/mL) was purchased from Amersham Life Science (Buckinghamshire, England). Castor oil, high-performance liquid chromatography (HPLC)-grade acetonitrile, and methanol were purchased from Nacalai Tesque, Inc. (Tokyo, Japan). All other chemicals were analytical grade and obtained from commercial sources.

CsA Assay

In the formulation studies, CsA was assayed using the modified HPLC method established by Novartis Pharma. A reversed phase YMC-Pack C_8 column (Model A-202, YMC Co., Ltd.) was used. The mobile phase was a mixture of acetonitrile:water:methanol:phosphoric acid (900:525:75:0.075). Its flow rate was 1.5 mL/min. The eluent was monitored by UV at 214 nm. The column oven temperature was maintained at 70 °C. Quantitation of peak area demonstrated excellent linearity (r^2 = 0.9999) over the CsA concentration range of interest.

Solubility Assay

Tween 80, HCO-60, and MYS-40 are nonionic surfactants that have been previously used in commercial ophthalmic products. Mixtures containing CsA and each surfactant (0.5 w/v %) were stored for 12 h to ensure equilibrium at 25 °C and then filtered through a 0.22-μm membrane filter. CsA concentrations in the filtrates were determined by HPLC. Effect of MYS-40 concentration on the solubility of CsA was determined at 25, 40, and 60 °C in the same experimental manner.

0.1 % ^{3}H-CsA Ophthalmic Formulation Preparation

^{3}H-CsA ophthalmic formulation (Aq-CsA) was prepared with the following procedure by first dissolving: CsA (0.0865 %) in a small amount of ethanol (0.1 %), and then MYS-40 (2 %) was added. The sample was well mixed until clarity returned. The CsA solution containing HPMC (0.3 w/v%), sodium dihydrogen phosphate (0.2 w/v%), and disodium EDTA (0.01 w/v%) was then adjusted to isotonicity (i.e., 287 mOsm) by the addition of sodium chloride. The pH was adjusted to 7. The needed volume of ^{3}H-CsA dissolved in an ethanol/toluene mixture was first dried in a nitrogen stream and then dissolved in the aforementioned aqueous solution containing CsA. It was stirred at 5 °C overnight to prepare Aq-^{3}H-CsA (37 MBq/mL).

To prepare a ^{3}H-CsA castor oil formulation (Oil-CsA), CsA (0.0865 %) was dissolved with stirring in a neat castor oil. ^{3}H-CsA was added in the same manner described above.

The ^{3}H-CsA o/w emulsion formulation (Em-CsA) was prepared as described (7). The oil phase consisted of CsA and castor oil. In brief, the CsA ethanolic solution was added to a mixture containing castor oil (1.25 %) and Tween 80 (1.0 %). Residual ethanol in the mixture was evaporated by a nitrogen stream. Pemulen® TR-2 (0.05 %) was dispersed in water and then mixed to obtain an o/w emulsion. This mixture was adjusted to isotonicity by the addition of glycerin and adjusted to pH 7.4. ^{3}H-CsA was added in the same manner as described previously.

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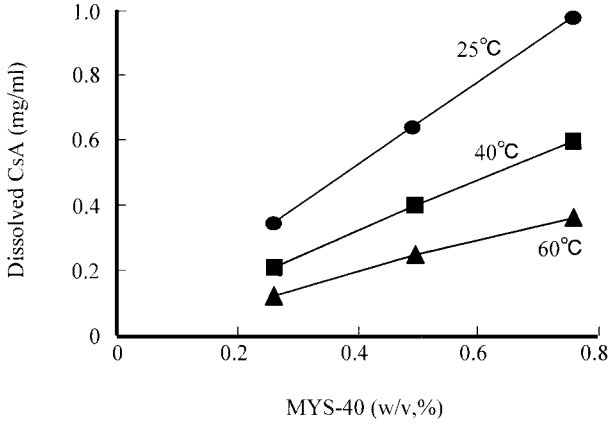

Fig. 1. Effects of MYS-40 concentration and temperature on the solubility of CsA in aqueous media.

Ocular Distribution Studies

The use and the treatment of rabbits in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male Japanese white rabbits weighing between 2 to 3 kg were used. A ^{3}H-CsA ophthalmic formulation (50 μL, 1850 kBq/eye) was topically instilled onto the right eyes. At 1, 4, and 24 h after instillation, the corneal epithelium, the corneal stroma and endothelium, the bulbar conjunctiva, the iris-ciliary body, the lacrimal gland, the Harder gland, aqueous humor, and the choroid-retina were dissected. Tissue CsA levels were calculated from the specific activity of ^{3}H-CsA and expressed as ng-eq/g tissue.

RESULTS

CsA Solubility In Ophthalmic Solutions

At 25 °C, the solubility of CsA in solutions containing one of three different surfactants (0.5 w/v %), MYS-40, HCO-60, and Tween 80, was 0.656, 0.554, and 0.455 mg/mL, respectively. CsA had the highest solubility in MYS-40 and, therefore, it was the best of the three surfactants for preparing clear solutions. Accordingly, MYS-40 was used throughout this study to prepare aqueous CsA solutions. The relationship between MYS-40 concentration and CsA solubility at 25, 40 and 60 °C is shown in Figure 1. It indicates that at each of these temperatures CsA solubility increased linearly. A 2 % MYS-40 aqueous solution was chosen to ensure that 0.1 %CsA was completely soluble in aqueous solution.

Ocular Distribution

Ocular distribution resulting from exposure to 0.1 % Aq-CsA was compared to those obtained with Oil-CsA and Em-CsA. The results in Table Ia and Ib show that most of the CsA administered in an Aq-CsA formulation distributed to ocular surface tissues such as the corneal epithelium, corneal stroma/endothelium, and bulbar conjunctiva. A small amount of CsA distributed to the lacrimal gland, the anterior and posterior segments. A trace amount of CsA was detected in the blood at all time points.

DISCUSSION

This study was undertaken to identify a CsA containing ophthalmic formulation that may be more desirable for de-

Table Ia. CsA Levels (ng-eq/g) in the Ocular Tissues after Single Instillation (50 μL) of Three CsA Formulations in the Rabbits

	Aq-CsA time (h)			Em-CsA time (h)		
Ocular tissue	1	4	24	1	4	24
Lacrimal gland	6.38 ± 8.09	3.06 ± 0.74	1.00 ± 0.42*	1.50 ± 1.46	1.21 ± 0.33	0.51 ± 0.13**
Harder gland	7.28 ± 10.86	1.70 ± 0.05	0.42 ± 0.15**	0.61 ± 0.20	0.59 ± 0.17	0.27 ± 0.07**
Bulbar conjunctiva	1373.80 ± 548.31**	542.73 ± 80.17**	27.83 ± 7.06	446.04 ± 164.73	287.60 ± 138.99*	19.70 ± 6.18*
Corneal epithelium	20234.65 ± 6348.76**	12247.78 ± 4775.28**	4932.76 ± 2143.96**	4952.95 ± 1348.05	5317.78 ± 1723.18	2662.46 ± 653.42
Corneal Stroma–endothelium	601.74 ± 485.45*	595.43 ± 230.85**	491.12 ± 114.00**	82.07 ± 38.14	175.45 ± 62.73	201.64 ± 69.33*
Aqueous humor	3.48 ± 1.35	1.22 ± 0.39**	1.45 ± 0.31	2.66 ± 0.62	0.86 ± 0.38	0.52 ± 0.15
Choroid–retina	2.28 ± 1.26	1.80 ± 0.48	1.54 ± 0.95	0.87 ± 0.34	0.85 ± 0.27	0.36 ± 0.08
Iris–ciliary body	3.56 ± 1.31**	5.44 ± 2.49**	23.95 ± 8.47**	1.76 ± 0.42	1.61 ± 0.58	10.07 ± 2.74
Whole blood	1.02 ± 0.64	0.31 ± 0.13	ND[b]	0.30 ± 0.06	0.21 ± 0.09	ND[b]
Blood plasma	0.29 ± 0.17	0.31 ± 0.21	ND[b]	—[a]	—[a]	—[a]

[a] Not assayed.
[b] ND: less than detection limit.
Each value represents mean ± SD of four experiments.
* $P < 0.05$; ** $P < 0.01$ vs. castor oil (Dunnett multiple comparison test).

Table Ib. CsA Levels (ng-eq/g) in the Ocular Tissues after Single Instillation (50 μL) of Three CsA Formulations in the Rabbits

Ocular tissue	Oil–CsA time (h)		
	1	4	24
Lacrimal gland	5.74 ± 7.94	5.22 ± 5.86	2.17 ± 0.99
Harder gland	4.56 ± 5.08	2.33 ± 2.17	1.64 ± 0.30
Bulbar conjunctiva	104.29 ± 30.53	88.46 ± 44.11	59.07 ± 33.31
Corneal epithelium	687.89 ± 427.28	744.26 ± 148.20	643.94 ± 150.51
Corneal stroma–endothelial	10.00 ± 4.20	10.51 ± 3.05	31.33 ± 7.02
Aqueous humor	2.73 ± 0.61	0.28 ± 0.05	ND[a]
Choroid–retina	2.38 ± 1.72	1.57 ± 0.92	0.92 ± 0.57
Iris–ciliary body	1.24 ± 0.34	0.38 ± 0.00	1.16 ± 0.27
Whole blood	ND[a]	0.19 ± 0.03	ND[a]
Blood plasma	ND[a]	0.14 ± 0.04	ND[a]

[a] ND: less than detection limit.
Each value represents mean ± SD of four experiments.

livering larger amounts of this immunosuppressant into ocular tissues. Such a route of application would be preferable because it substantially reduces the likelihood of adverse effects that result from CsA systemic administration. Prior to this study, it was not possible to envisage the use of an aqueous formulation that also had no irritating ocular side effects. The only formulations that could be used to dissolve CsA required an oil base. However, these formulations (i.e., with up to 1% CsA) did not result in much ocular tissue CsA accumulation. Furthermore, an oily formulation for ophthalmic use is undesirable because it causes vision blurring and is irritating. We have shown that the level of CsA accumulation by most ocular tissues can be markedly enhanced by applying an aqueous CsA formulation that contains the nonionic surfactant MYS-40. High CsA concentrations were observed in the corneal stroma–endothelium at all time points after a single topical instillation of the newly formulated aqueous solution (Aq-CsA) in comparison with those for Oil-CsA and Em-CsA. At 1-h post instillation, CsA levels for Aq-CsA were 60.2 and 7.3 times higher than that for Oil-CsA and Em-CsA, respectively (Table I). This pattern was also observed in the bulbar conjunctiva. CsA exhibited the following penetration order: Aq-CsA > Em-CsA > Oil-CsA.

The area under the curve (AUC_{0-12}) and AUC ratio, which were calculated using AUC (Oil-CsA) as a control, are shown in Table II. The AUC for Aq-CsA in the corneal stroma-endothelium, which is the target tissue for preventing corneal allograft rejection after corneal transplantation surgery, was 28.5 and 3.1 times higher than those for Oil-CsA and Em-CsA, respectively.

Table II. Comparison of AUC_{0-12} for the Corneal Stroma–Endothelium, Bulbar Conjunctiva, and Lacrimal Gland after Single Instillation of 0.1 % Cyclosporine A in Three Different Formulations in the Rabbits

Ocular tissue	Oil-CsA		Aq-CsA		Em-CsA	
	AUC	Ratio	AUC	Ratio	AUC	Ratio
Corneal stroma–endothelium	454.2	1	12962.1	28.5	4198.2	9.2
Bulbar conjunctiva	1816.6	1	9267.3	5.1	4396.5	2.4
Lacrimal gland	93.2	1	58.0	0.6	22.0	0.2

AUC_{0-12}: ng-eq · h/g. Each ratio represents AUC (each formulation)/AUC (Oil–CsA).

It appears that CsA penetration into the ocular surface tissues is affected by the release rate of CsA from its carrier in the dispersion medium. In an aqueous medium containing a nonionic surfactant, micelles are formed and CsA was dissolved in them. The size of micelles is much smaller than that of emulsion droplets, which is 200 nm in mean diameter (7). Accordingly, much more CsA can be released from micelles for drug penetration because they have a much larger surface area, enabling them to bind much more drug. Another impediment limiting drug penetration is that CsA has a strong affinity for lipophilic vehicles, such as vegetable oils, because their partition coefficient is 6. Therefore, CsA release is poor from oily vehicles and o/w emulsion. Perry *et al.* determined the cornea CsA levels in patients, who were topically treated with 0.5 % CsA every 15 min for one hour before corneal surgery (8). The mean cornea CsA level was 3679 ng/g in 9 patients. In our study, the maximal CsA corneal level reached was 2112 ng/g (total cornea) at 1 h after only a single administration of 0.1 % aqueous CsA solution. This value is equal to the sum of the normalized CsA content on a wet weight basis in the corneal epithelial, stromal and endothelial layers. This result suggests that the CsA concentration in the ophthalmic solution could be reduced below 0.1% to achieve the same extent of penetration as in patients. Alternatively, even greater penetration may be possible by using the 0.1 % aqueous CsA solution.

CONCLUSIONS

A cyclosporine aqueous ophthalmic solution that uses polyoxyl 40 stearate as a solubilizer for CsA has been designed. An ocular pharmacokinetic study using ^{3}H-CsA showed that the distribution of CsA in ocular tissues, such as cornea, bulbar conjunctiva, and lacrimal gland, after topical instillation of the aqueous formulation was superior to that after instillation of an o/w emulsion and an oily formulation. We suggest that this aqueous CsA ophthalmic formulation will be clinically useful in the treatment of immune-mediated ophthalmic diseases.

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