Local Cyclosporine Therapy for Experimental Autoimmune Uveitis in Rats

Robert B. Nussenblatt, MD; William J. Dinning, FRCS, MRCP; Leslie S. Fujikawa, MD; Chi-Chao Chan, MD; Alan G. Palestine, MD

● **The use of locally applied cyclosporine was investigated in the retinal S-antigen–induced experimental autoimmune uveitis (EAU) model in Lewis rats. A 2% cyclosporine solution applied topically four times a day for 14 days effectively prevented the expression of EAU. This treatment, however, produced circulating cyclosporine levels in the therapeutic range. Lower concentrations of cyclosporine applied topically did not produce therapeutic levels and were not capable of reliably preventing disease. Intraocular levels of cyclosporine, measured by radioimmunoassay, were extremely low and outside the accepted therapeutic range. Intravitreal cyclosporine therapy appeared to protect eyes from EAU, without producing significant circulating cyclosporine levels. These findings show that, in its present form, cyclosporine in oil is not an efficacious topical therapy. Therefore, a local cyclosporine preparation with enhanced penetration into the globe may be a practical approach to therapy in the future.**

(*Arch Ophthalmol* 1985;103:1559-1562)

Experimental autoimmune uveitis (EAU) can be dependably induced by immunization of lower mammals and nonhuman primates with the retinal S antigen.[1,2] This model has many characteristics similar to certain human uveitic conditions.[2] The immunopathology, localized to the eye and, in some species, to the pineal gland,[3] appears to be T-cell mediated,[4] although the susceptibility of various inbred strains is related to the choroidal mast cell numbers.[5] We have previously reported the effective abrogation of the disease manifestations of this animal model with systemically administered cyclosporine,[6] an agent with unique anti-T-cell characteristics.[7] Further, we have also reported the beneficial effect of cyclosporine in the treatment of severe intermediate and posterior endogenous uveitis in patients who had failed to respond to corticosteroid and/or cytotoxic agents.[8] The systemic administration of this agent is associated with well-recognized adverse reactions, with renal toxic effects and hypertension being the most common serious complications noted in our patients.[9] We present herein our experience in the topical and intracameral administration of cyclosporine in Lewis rats, its effect on the development of experimental autoimmune uveitis, and its penetration into the eye.

Accepted for publication June 10, 1985.

From the Clinical Branch, National Eye Institute, National Institutes of Health, Bethesda, Md.

Reprint requests to National Eye Institute, National Institutes of Health, Bldg 10, Room 10N222, Bethesda, MD 20205 (Dr Nussenblatt).

MATERIALS AND METHODS

Female Lewis rats, each 6 weeks of age and weighing approximately 200 g, were used for this series of experiments. Animals receiving topical and systemic medications were immunized in both hind foot pads with a total of 50 μg of bovine S antigen, prepared as described elsewhere,[10] mixed with an equal portion of complete Freund's adjuvant augmented with H37 *Mycobacterium tuberculosis* to a concentration of 2.5 mg/mL. Animals receiving intracameral cyclosporine therapy were immunized with 30 μg of bovine S antigen prepared and mixed in the same fashion as above.

Cyclosporine Therapy

Topical Therapy.—A 2% cyclosporine solution in olive oil was the stock solution. Lower concentrations of the drug were obtained by diluting the stock solution with olive oil. Animals were treated topically with 2%, 1%, 0.5%, and 0.2% cyclosporine. The frequency of administration and whether both eyes or only one eye were treated are mentioned in Table 1. For the determination of cyclosporine penetration into the eye, only one drop (50 μL) of the concentrations tested was placed onto the eye.

Intracameral Administration.—Using the stock 2% cyclosporine solution, 40 μL (800 μg) was injected intravitreally 11 days after S-antigen immunization. Other rats received intravitreal olive oil. This was performed using the operating room microscope for visualization and a 30-gauge needle.

Systemic Administration.—Animals received 10 mg/kg/day of cyclosporine intramuscularly for 14 days. Cyclosporine penetration into the eye was evaluated by freezing the tested eyes for immunofluorescent staining or for dissection of the inner contents in order to obtain cyclosporine levels. Cryostat sections, 4 μm thick, of eyes were washed with phosphate-buffered saline and incubated in a moist chamber for 30 minutes at room temperature with sheep anticyclosporine antibody (1:2) followed by fluoresceinated rabbit anti-sheep IgG (1:10). Washed sections were mounted in polyvinyl alcohol resin and viewed with an epi-illumination fluorescence microscope (Leitz). Eyes were frozen after enucleation at the level of the pars plana, and the intraocular contents (lens, vitreous, retina, and choroid) were then extruded. These were homogenized in 1 mL of 95% alcohol, spun at 2,000 rpm for ten minutes at 4 °C. The soluble fraction was then assayed for its cyclosporine content using a radioimmunoassay.

Grading of Ocular Inflammatory Disease

This was performed in a masked fashion using a modification of the grading system described by Wacker and associates,[10] which was for guinea pigs. In this system for the evaluation of posterior segment disease in the rat, the grading is as follows:

Table 1.—Cyclosporine Therapy for EAU*

Initiation and Duration of Treatment After S-Antigen Immunization	Treatment Schedule and % of Cyclosporine Solution	OU†	OD†	OS†	Statistical Significance‡
		No Treatment			
None (control)	Untreated	0/8 (2.95)§	...	...	...
		Both Eyes Treated With IM Injection			
Days 0-14	2 mg qd	8/8 (0)	...	...	$P < .00008$
		Group A: OU Treated With Drops			
Days 0-14	2, qid	7/8 (1)	...	...	$P < .0001$
	0.5, qid	4/6 (1.6)	...	...	$P < .015$
	0.2, qid	4/8 (2.75)	...	...	$P < .04$
Days 7-14	1, qid	4/8 (1.75)	...	...	$P < .04$
	0.5, qid	0/6 (2.9)	...	...	NS
	0.2, qid	0/8 (1.8)	...	...	NS
		Group B: OD Only Treated With Drops			
Days 0-14	2, bid	...	4/5 (1)	4/5 (1)	NS
	0.5, qid	...	3/4 (2)	3/4 (2)	NS
	0.2, qid	...	1/4 (3.3)	1/4 (2.7)	NS
Days 7-14	1, qid	...	0/4 (3.0)	0/4 (3.4)	NS
	0.5, qid	...	2/4 (2)	2/4 (2)	NS
		Group C: Intravitreal Injection in OD Only			
Day 11	80 μg	...	4/4 (3.5)	4/4 (3)	NS
	500 mg	...	6/8 (0.75)	1/8 (2.6)	$P < .02$
	Vitreous puncture with olive oil	...	3/3 (2.5)	3/3 (3)	NS

*Abbreviations are as follows: EAU, experimental autoimmune uveitis; NS, not significant; IM, intramuscular; qd, every day; qid, four times a day; bid, twice a day; OD, right eye; OS, left eye; OU, both eyes.

†Values given are number of normal eyes/total number of eyes.

‡One-tailed Fisher's exact test performed. For systemic therapy and group A, treatment groups compared with untreated group. For groups B and C, comparisons are made between right and left eyes.

§Inflammatory index (see text).

0, no evidence of inflammatory disease; trace (0.5+), architecture of retina grossly intact. Areas of focal destruction were 1+, focal areas of destruction with marked dropout of photoreceptors; 2+, small exudative retinal detachment with larger destruction, mild to moderate number of cells in vitreous; 3+, retinal architecture beginning to be lost, larger exudative retinal detachment, moderate to large number of cells in vitreous; and 4+, total destruction of retinal architecture.

RESULTS

The results of topical cyclosporine therapy can be seen in Table 1. All animals were killed 14 days after immunization. Good protection from the manifestation of EAU could be obtained by treating both eyes of rats four times a day for 14 days with a topical solution containing 20 mg/mL of cyclosporine beginning on the day of immunization. Serum samples taken from these rats four hours after topical cyclosporine instillation, however, demonstrated high circulating cyclosporine levels, the mean being 285 ng/mL. A lower concentration of the topical cyclosporine solution (0.5%) was capable of protecting many eyes treated, if begun on the day of immunization, while the 2 mg/mL (0.2%) solution was less effective and a higher inflammatory index was noted. To further evaluate the potential local effect of cyclosporine on EAU, some rats were treated topically only in the right eye. Animals receiving drops to one eye appeared to have only partial protection. The animals receiving a 2% solution twice a day for 14 days only to the right eye had a mean cyclosporine plasma level of 108 ng/mL. At lower concentrations protection was not consistently observed.

The efficacy of topical cyclosporine therapy appeared to be considerably less when begun seven days after S-antigen immunization if topical cyclosporine concentrations were those that did not result in consistently detectable circulating levels. Intravitreal injection of 800 μg of cyclosporine 11 days after S-antigen immunization appeared to alter the expression of EAU (Table 1) in the eye receiving the cyclosporine. In a series of experiments in which cyclosporine was placed only in one eye, the treated eye manifested no disease or only minimum inflammatory changes with retention of the retinal architecture (Fig 1, left), in contrast with the untreated eye, where inflammation was seen (Fig 1, right). Mean circulating plasma cyclosporine levels three hours after injection into the vitreous were 60 ng/mL, while at four hours the levels were low (26 ± 8 ng/mL). Serum cyclosporine levels were not detectable at the time of death three days after instillation, however, suggesting that a local therapeutic effect had taken place.

The intraocular contents of cyclosporine-treated eyes were evaluated for the presence of cyclosporine using two routes of administration (Table 2). The topical application of one drop of cyclosporine at two concentrations led to levels in the intraocular contents of those eyes that were extremely low, indeed at the level approaching the sensitivity of the radioimmunoassay. Somewhat higher concentrations were noted when one drop of the 20 mg/mL (2%) solution was used as opposed to the 2 mg/mL (0.2%) preparation.

A dose-response curve was obtained with the instillation of 80 or 800 μg of cyclosporine intracamerally. Potentially significant therapeutic levels were still found in the eye several days after the injection of the higher amount.

Immunofluorescence staining of sections of eyes receiving topical cyclosporine demonstrates a clear pattern (Fig 2). The corneal epithelium appears to stain brightly within a few minutes of cyclosporine application. Staining of the internal structures of the eye was insignificant.

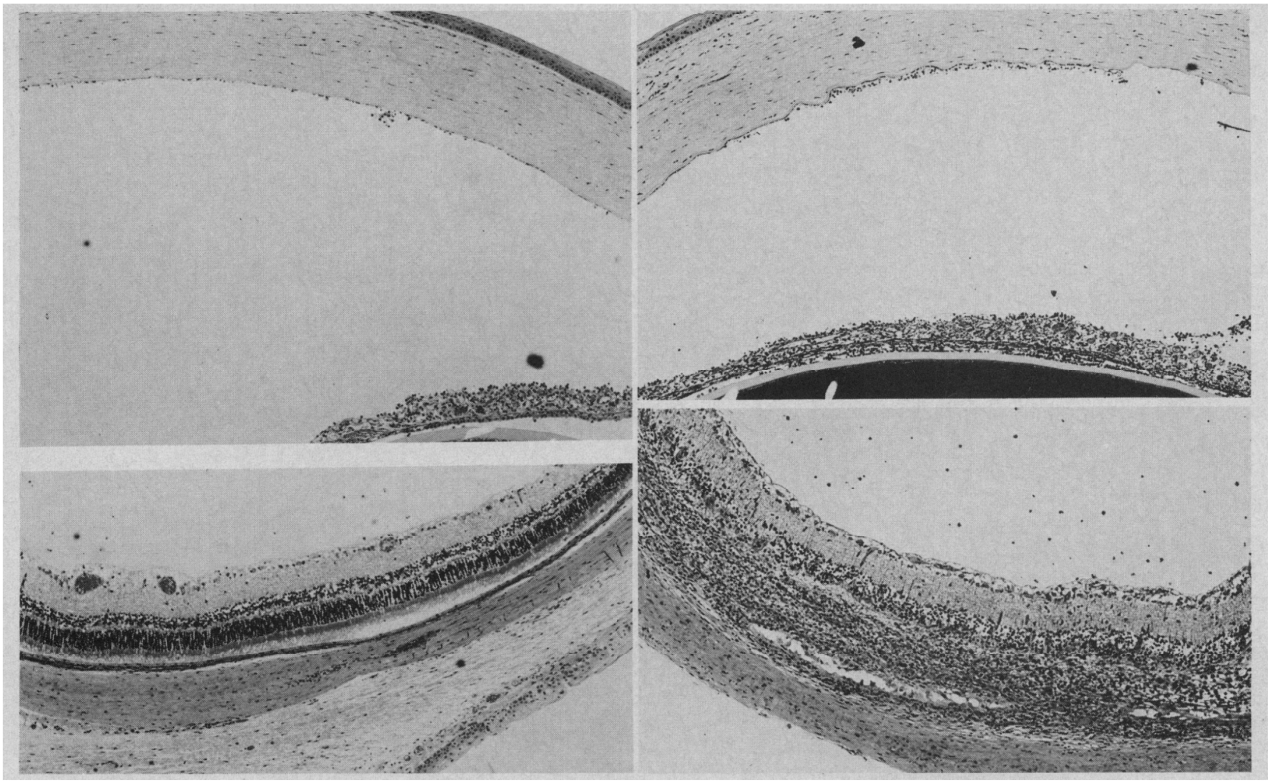

Fig 1.—Left, Right eye of Lewis rat that received 800 μg of cyclosporine intravitreally 11 days after S-antigen immunization. At 14 days after immunization, retinal architecture is grossly intact. Only occasional inflammatory cells in anterior chamber, iris, and vitreous are visible. Right, Left eye of same rat as in Fig 1, left. This eye received no therapy. At 14 days, severe anterior and posterior inflammatory response is evident, with destruction of retinal architecture (×90).

Table 2.—Rat Vitreous Cyclosporine Levels After Local Administration

Cyclosporine Administration	Dosage	Cyclosporine Levels in Vitreous After Application,* mg/mL				
		1 hr	4 hr	24 hr	48 hr	96 hr
Topical	2% solution	18	7	19	7	. . .
	0.2% solution	3	3	3	3	. . .
Intravitreal	800 μg	ND†	580	390	ND	160
	80 μg	ND	60	80	ND	30

*Mean of at least four eyes per group.
†ND indicates not done.

Faint staining of the iris, ciliary body, and retina was seen in some sections one to four hours after topical instillation. No increase in the staining pattern, however, could be observed with time. Of interest was the intense staining of the posterior sclera.

COMMENT

We report herein that the effective control of EAU can be accomplished by the topical administration of cyclosporine in doses adequate to produce significant plasma levels. The systemic administration of cyclosporine effectively prevented the manifestations of EAU, even after cells immunoreactive to the S antigen can be demonstrated.[6] These animal studies and human studies both suggest that the efferent arm of the immune system was particularly affected. The question as to whether local administration of cyclosporine would affect ocular inflammation raises not only practical but also theoretic considerations.

Cyclosporine appears to interrupt T-cell activation at an early point at a state of antigen presentation.[7] Dos Reis and Shevach,[11] in an in vitro model using guinea pigs, demonstrated that cyclosporine appeared to block IL-2 production and responsiveness

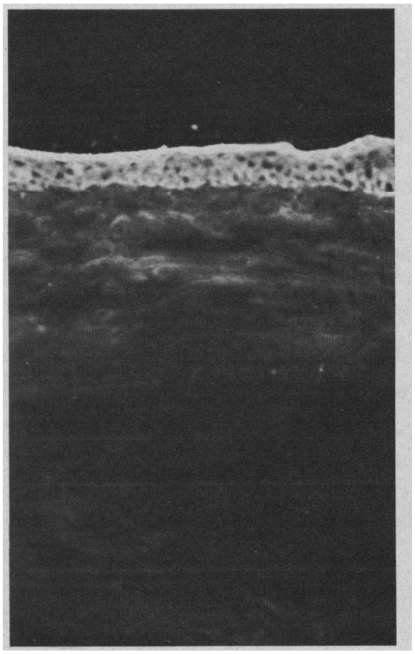

Fig 2.—Immunofluorescent localization of cyclosporine in cornea 30 minutes after topical application of 2% solution. Corneal epithelium stained brightly (×200).

and, depending on the stage of the T cell, appeared to directly block the induction of IL-2 receptors. The addition of exogenous IL-2 was variable in its capacity to overcome cyclosporine inhibition, this depending on the T-cell stimulus as well as the stage of the differentiation. Further, Kaufmann and colleagues,[12] employing hybridomas devoid of functional IL-2 receptors in an in vitro system, obtained results suggesting that cyclosporine action on T cells could be through the interference of antigen binding to antigen receptors, with a resultant blockade of the lymphokine cascade. Further, it has been shown that T cells bearing IL-2 receptors are relatively immune from the effects of cyclosporine.[7]

We surmised that effective control of severe ocular inflammation by systemically administered cyclosporine was due to a central effect of the agent, but primarily on the efferent arm of the immune response. We have reported the presence of intraocular T cells bearing the TAC (IL-2) receptor,[13] a sign of T-cell activation. We therefore suggested that in endogenous uveitis a rather rapid influx of immunoreactive cells into the eye was occurring, therefore making it possible for cyclosporine to rapidly interrupt the cycle of immune recruitment.

The effectiveness of intracameral cyclosporine administration would suggest that the final activation of the recruited T cells may be a local (ocular) phenomenon. This could explain the profound inflammatory ocular response one can observe with little evidence of a systemic one in many uveitic conditions. Another potential mechanism may be cyclosporine's effect on ocular tissue involved in the localization to the eye of the immune response. It has now been demonstrated that the localized expression of Ia on vascular endothelial cells is associated with T-cell-mediated disorders, such as experimental allergic encephalomyelitis.[14] Since γ-interferon is the most effective natural stimulant for Ia expression, it is possible that cyclosporine's prevention of the release of lymphokines from T cells might alter the continued expression of Ia on the ocular vascular endothelium, thereby interrupting the proposed "homing" mechanism of immunoreactive cells to a site of inflammation.

The immunofluorescent staining pattern deserves comment. This showed that cyclosporine quickly coated the corneal epithelium. The presence of cyclosporine intraocularly after topical application, however, was minimal at best. Weak staining of the iris and retina could be seen on occasion, but this was not the case for all eyes. This observation was supported by the use of the radioimmunoassay, which also failed to detect significant intraocular levels of cyclosporine. We do know from our human experience that cyclosporine can be detected in the eye after systemic administration,[15] but these observations were made in eyes with a nonintact blood-aqueous barrier. It may be that the lipophilic structure of cyclosporine does not permit it ready access into the eye. Our findings support those observed by Mosteller and colleagues.[16] They noted that cyclosporine collected in high concentrations in the rabbit cornea, while low amounts were measured in the aqueous humor. This lack of penetration would explain our inability to reliably protect rats from EAU with topical cyclosporine therapy.

As we have noted, topical therapy seemed predictably effective only if serum cyclosporine levels entered what is considered the therapeutic range of 50 to 300 ng/mL. Although this observation would support the notion that the action of cyclosporine is central, the subsequent finding that intracameral cyclosporine effectively protected the eye in which it was injected raises the possibility of a peripheral effect as well. The levels in the eye receiving cyclosporine indicated a therapeutically acceptable range. Therefore, locally applied cyclosporine may potentially be useful in certain human uveitic conditions, particularly those endogenous disorders with no systemic associations. The need for greater drug penetration into the eye, however, still has to be addressed. It may be that a change in the vehicle, such as to liposomes, may permit a higher concentration of cyclosporine to enter the eye. The development of this methodology will permit the testing of many theoretic considerations and will also be of practical import.

The cyclosporine and radioimmunoassay used in this investigation were supplied by Sandoz, Ltd, Basel, Switzerland.

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