

## Penetration of 2% cyclosporin eyedrops into human aqueous humour

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**SUMMARY** The penetration into the eye and the systemic absorption of 2% cyclosporin eye drops were determined by polarisation immunofluorescent assay of cyclosporin in the aqueous humour and plasma of 30 patients at the time of cataract surgery. The results were then compared with the corresponding results after oral administration at a dosage of 5 mg/kg/day to three further patients. The maximum intraocular concentration (24 (SD 9) mg/l) was achieved four hours after topical administration. This level was slightly less than that found in aqueous humour (28 (SD 10) mg/l) 12 hours after systemic administration of the drug at a dosage of 5 mg/kg/day. Both these levels are below the minimum therapeutic level (50-100 mg/l). Topical application of cyclosporin A did not produce detectable levels in plasma at any time. These results indicate that the efficacy of cyclosporin A in the control of intraocular inflammation is attributable to its systemic immunoregulatory effect rather than to the local ocular effect.

Cyclosporin A is a drug which exerts a potent selective immunosuppressant effect on certain functions of T lymphocytes in the cell-mediated immune response.<sup>1</sup> It has proved to be very effective in the treatment of cases of severe endogenous uveitis refractory to other drugs such as corticosteroids and other immunosuppressants (azathioprine, chlorambucil, and others).<sup>2</sup> The systemic administration of cyclosporin A even at dosages as low as 5 mg/kg/day is, however, associated with some well known side effects, in particular nephrotoxicity, arterial hypertension, gingival hypertrophy, and hypertrichosis.<sup>3</sup>

It is not yet known whether the efficacy of cyclosporin A is due to its systemic action on the immune system or whether it is due to its regulatory action on immunoreactive cells at the local ocular level. In a large number of animal experiments cyclosporin A has proved effective in prolonging the survival time of corneal grafts whether administered via the subconjunctival,<sup>4</sup> retrobulbar,<sup>5</sup> intramuscular,<sup>6</sup> or topical<sup>7</sup> routes. This suggests that the effect of cyclosporin A is due principally to a local immunoregulatory effect at the ocular level rather than to a systemic effect.

Local administration of cyclosporin A as 2% eye-

drops has also proved to be effective both in preventing and in controlling the progression of experimental autoimmune uveitis induced by retinal S-antigen, provided always that therapeutic plasma levels are achieved.<sup>8</sup> This suggests that the therapeutic action of cyclosporin A is due to its systemic immunoregulatory effect rather than to its local ocular effect.

The aim of the present study was to determine the extent of penetration of cyclosporin A into human aqueous humour and serum after local ocular administration of 2% eyedrops, since this information, which is not yet known, is essential in order to establish the mechanism of action of this drug on ocular inflammation.

### Material and methods

A 2% solution of cyclosporin in olive oil was used for topical administration.

After a detailed explanation to each patient of the purpose of the study, two drops (100 µl) of 2% cyclosporin solution (20 g/l) were applied to one eye every six hours—the treatment starting 72 hours before cataract surgery—to a total of 30 patients aged between 59 and 71 years.

Aqueous humour (0.15–0.20 ml) was obtained by aspiration of the anterior chamber with a 25-G needle

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at the following times after the final topical application of the drug: ½ an hour in five patients, 2 hours in five patients, 3 hours in five patients, 4 hours in five patients, 5 hours in five patients, and 12 hours in five patients. All these times corresponded to the start of cataract surgery.

A specimen of plasma was obtained at the same time as the aqueous humour, and both specimens were then assayed by polarisation immunofluorescence (Abbott) for cyclosporin A content.

Three further patients were given a once daily oral dose of 5 mg/kg/day of cyclosporin A, the treatment starting one week before cataract surgery. The specimens of aqueous humour and plasma were taken simultaneously at the time of surgery and 12 hours after oral administration of the drug, and the level of the drug in both specimens was determined.

## Results

The individual plasma and intraocular concentrations of cyclosporin A after topical therapy with 2% cyclosporin are shown in Table 1. The peak concentration in aqueous humour (24 SD 9) mg/l was achieved four hours after application of the solution. The level then declined progressively until at 12 hours after the final application cyclosporin A was no longer detectable. At no stage was the drug identified in the plasma.

The three patients who received 5 mg/kg/day of cyclosporin A orally had plasma levels of 247, 161, and 210 mg/l respectively 12 hours after administration. The corresponding levels in aqueous humour

were 28, 20, and 36 mg/l respectively (mean 28 (SD 10) mg/l) (Table 2). These levels are similar to those found four hours after topical administration of the 2% solution of cyclosporin A.

## Discussion

Cyclosporin A is a neutral hydrophobic cyclic peptide. The corneal epithelium is also hydrophobic and is therefore easily penetrated by lipid soluble substances such as cyclosporin A. The corneal stroma, in contrast, is hydrophilic and therefore does not permit the passage of a hydrophobic drug such as this. Topically applied cyclosporin A easily penetrates the corneal epithelium and accumulates in the stroma, only very small amounts passing into the aqueous humour.

This was demonstrated by Mosteller and associates,<sup>9</sup> who applied a total topical dose of 3 mg/kg/day of cyclosporin A to rabbits. This resulted in levels of more than 900 mg/l in corneal tissue three hours later, whereas the concentration in aqueous humour was less than one-thirteenth of this.

Topical administration of cyclosporin A has been proposed as an alternative to systemic in the treatment of uveitis which would avoid the appearance of side effects (nephrotoxicity, arterial hypertension, etc.). This idea appeared to be supported by a number of animal experiments, in all of which topical application of cyclosporin A achieved plasma levels within the range regarded as therapeutic (50–300 mg/l),<sup>10</sup> even though this level was not reached in the aqueous humour. The application of 2% cyclosporin

Table 1 Concentration of cyclosporin A in human aqueous humour following application of 2% cyclosporine eyedrops

No. of hours after application	Number of specimens	Concentration in aqueous humour (mg/l)	Concentration in plasma (mg/l)
½	5	0	0
2	5	6 (SD 3)	0
3	5	12.5 (SD 5)	0
4	5	24 (SD 9)	0
5	5	16 (SD 7)	0
12	5	0	0

Table 2 Concentration of cyclosporin A in aqueous humour following once daily administration of 5 mg/kg/day orally

No. of hours after application	Number of specimens	Concentration in aqueous humour (mg/l)	Concentration in plasma (mg/l)
12	1	28	247
12	1	20	161
12	1	36	210
		28 (SD 10)	204 (SD 43)

Table 3 Comparison of the levels of cyclosporin A in aqueous humour and plasma in relation to different topical administration protocols in various species

Study	Species	Type and route of administration	Dose of cyclosporine (mg/kg/day)	Hours since administration	Concentration in aqueous humour (mg/l)	Concentration in plasma (mg/l)
Mosteller <i>et al.</i> <sup>9</sup>	Rabbit	10% ointment	3	4	62	75
Nussenblatt <i>et al.</i> <sup>8</sup>	Rat	2% eyedrops	40	4	–	285
Bell and Hunisett <sup>11</sup>	Rabbit	1% eyedrops	2.5	–	27	116
Present study	Man	2% eyedrops	0.11	4	24	0

eye drops to rats by Nussenblatt and associates,<sup>8</sup> for example, resulted in plasma levels of 285 and 108 mg/l following doses of 40 and 10 mg/kg/day respectively. These concentrations of the drug are effective as prophylaxis and therapy of experimental autoimmune uveitis (EAU) induced by retinal S-antigen. Topically applied cyclosporin A was effective against EAU only if therapeutic levels (50–300 mg/l) were reached, but in such cases it was effective even with very low, subtherapeutic, intravitreal levels (19 mg/l). This observation suggests that the principal mechanism of action of cyclosporin A in controlling severe ocular inflammation is not so much its local ocular effect but rather its systemic effect on the immune system, in that by blocking the production of interleukin 2 (IL-2) it interrupts the entire lymphokine cascade. However, the demonstrated efficacy of intracamerally administered cyclosporin A against EAU suggests that the drug also exerts a local ocular effect.

The topical administration of 3 mg/kg/day of cyclosporin A to rabbits by Mosteller and associates<sup>9</sup> resulted in plasma and aqueous humour levels of 75 and 62 mg/l respectively four hours later.

Bell and Hunisett<sup>11</sup> applied a topical dosage of 2.5 mg/kg/day of cyclosporin A to rabbits and found levels of 116 mg/l in plasma and 27 mg/l in the aqueous humour. This plasma level produced a level of 18 mg/l in the aqueous humour of the contralateral eye.

Given that the ocular surface area of rats and rabbits is incomparably larger in relation to body weight than it is in man, it is not surprising that the topical administration of cyclosporin A should result in therapeutic plasma levels in these animals.

The topical therapy protocol which we used in humans was very similar in terms of concentration, dose, and frequency of administration of cyclosporin A to that used in previous animal studies.<sup>8,11</sup> This meant that the total dose of cyclosporin A for a 70 kg individual was only 0.11 mg/kg/day; hence it is not surprising that cyclosporin A was not detectable in plasma. The peak concentration in aqueous humour (24 (SD 9) mg/l) was reached four hours after administration. These low intracameral levels together with the absence of therapeutic plasma concentrations (0 mg/l) account for the fact that 2% cyclosporin eyedrops are ineffective either in endogenous posterior and intermediate uveitis or even in anterior uveitis, this fact being confirmed by our own clinical experience. Table 3 shows a comparison of the levels of cyclosporin A in aqueous humour and plasma in various species in relation to different administration protocols.

The low intraocular levels of cyclosporin A produced by either topical or systemic administration

contrast with the high concentrations of the drug in other tissues such as lymph nodes, for example, in which the level may be 10 times higher than in plasma.<sup>12</sup>

The use of a different vehicle (for example, liposomes) or clinical trials with solutions of higher concentrations (for example, 10%) may produce therapeutic levels (>50 mg/l) in the aqueous humour and thus make it possible to establish precisely what local immunoregulatory effect is exerted by cyclosporin A in endogenous anterior uveitis.

The ratio (0.14 (SD 0.03)) of the level of cyclosporin A found in human aqueous humour to that in plasma 12 hours after systemic administration of low doses (5 mg/kg/day) was lower than that found by Palestine and associates<sup>13</sup> (0.40 (SE 0.24)) 24 hours after local administration of the drug. The actual level was slightly higher than that found four hours after topical administration. This fact can be regarded as strong evidence for the hypothesis that the principal action of cyclosporin A in endogenous uveitis is a systemic immunosuppressant effect rather than a local intraocular immunoregulatory effect. We consider that larger scale studies are required in order to determine precisely the nature of the immunoregulatory action of cyclosporin A at the local and systemic levels in the treatment of intraocular inflammation.

We conclude that topical administration of 2% cyclosporin eyedrops produces subtherapeutic levels of cyclosporin in the aqueous humour and undetectable levels in plasma. Systemic administration of cyclosporin A at a dosage of 5 mg/kg/day produced subtherapeutic levels in aqueous humour, but therapeutic levels in plasma. We conclude from this investigation that the principal action of cyclosporin A on intraocular inflammation is a systemic one on the immune system rather than a local immunoregulatory action at the ocular level, and therefore that the use of 2% cyclosporin eyedrops cannot be regarded as a satisfactory treatment of refractory anterior uveitis.

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