The use of combined intravenous pulse methylprednisolone and oral cyclosporin A in the treatment of corneal graft rejection: a preliminary study

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CLINICAL STUDY

Abstract

Purpose **Oral cyclosporin A used in addition to high-dose intravenous pulse methylprednisolone has been shown to have an adjunctive effect in reversing the rejection of liver and renal transplants. The aim of this prospective study was to evaluate the benefits and risks of this combined drug therapy in acute corneal graft rejection.**

Methods **Eleven patients with acute corneal graft rejection received the combined regimen of a single pulse of intravenous methylprednisolone (500 mg) and a low dose of oral cyclosporin A (to maintain a trough blood level of 100–200 μg/l).**

Results **At a mean follow-up of 16.5 months (range 8–22 months) from the presentation of the graft rejection, reversal of graft rejection was achieved in 10 of 11 cases (90.9%). No recurrence of graft rejection was encountered during the study period. One patient developed a duodenal ulcer, which healed after medical treatment. No other complications were encountered.**

Conclusions **The high efficacy and low risk of the combined regimen demonstrated in this preliminary study call for a larger-scale prospective double-masked study to confirm the value of this treatment protocol.**

Key words Corneal graft rejection, Cyclosporin A, Intravenous methylprednisolone

Immune-mediated graft rejection is the leading cause of graft failures after the immediate post-operative period, accounting for up to 34% of failures.[1,2] All three layers of the cornea can be rejected either alone or in combination,[3] but the endothelium is the most important layer to be affected by rejection. The corneal endothelium is crucial in maintaining the normal physiology, and thus the clarity and function, of the cornea. Graft rejection can result in significant and irreversible endothelial cell loss.[4] If rejection is not promptly treated, the irreversible damage to the endothelium may be severe enough to leave the cornea permanently oedematous and non-transparent, resulting in graft failure and poor vision.

Corticosteroids remain the mainstay of treatment for graft rejection. It was shown that topical steroid alone had an unacceptably low success rate in reversing corneal graft rejection.[5] Amongst systemic steroid regimes, it was shown that a single pulse intravenous regimen was more effective than oral prednisolone in arresting corneal graft rejection.[6] Furthermore, it was demonstrated that grafts treated with pulsed therapy were statistically less likely to have a rejection episode in the future, compared with those treated with oral steroids.[6] A preliminary study showed that the success rate of reversing graft rejection by a single 500 mg pulse of intravenous methylprednisolone, with hourly topical steroid, was 80%.[7] Pulse therapy also avoids the complications of prolonged oral corticosteroid therapy. There is, however, no apparent benefit from administering a second pulse of steroid.[8] The exact mechanism of action of pulse steroid is not known, but is believed to be a selective depletion of lymphocytes, namely the $T_{helper/inducer}$ subpopulations.[9]

As corticosteroids are not always effective,[7] and serious complications of their use can also occur, much effort has been made to find alternative, or adjunctive, therapies to control corneal graft rejection. One of these is cyclosporin A. Cyclosporin A is a powerful immunosuppressive agent derived from the fungus *Tolypocladium inflatum* Gams, first reported by Borel *et al.* in 1976.[10] It appears to act at the early stages of antigenic sensitisation. Its exact mechanism of action is unclear, but probably involves inhibition of antigen presentation and subsequent lymphokine production.[11] The therapeutic value of cyclosporin A as a prophylactic

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immunosuppressive agent is well established.[12,13] In corneal transplant, the value of systemic cyclosporin A in preventing graft failure due to rejection in high-risk keratoplasties has also been well proven.[14–16] Regarding the reversal of established graft rejection, cyclosporin A is traditionally believed to be only marginally effective and is dose dependent.[17–19] However, in 1983 Margreiter *et al.*[20] reported a pilot study in which a series of 9 patients with acute cadaveric renal allograft rejection were given oral cyclosporin A when their rejection was found to be unresponsive to high-dose steroids. The grafts in all 9 patients survived. This prompted their group to proceed to a prospective randomised trial.[21] Even though the results of this latter trial were not as dramatic as those of the pilot study, with only 5 of 12 (41.7%) rejections reversed by the addition of oral cyclosporin A, the additive value of systemic cyclosporin A in the treatment of acute graft rejection was becoming convincing. One additional observation made during their trials was that the beneficial effect of oral cyclosporin A seemed to appear only hours after its commencement, suggesting that a low serum level of cyclosporin A may already be effective.

It remains to be seen whether the addition of systemic cyclosporin A to the single intravenous pulse steroid regimen offers any extra benefits in the management of acute corneal graft rejections. Our preliminary study presented here was an initial attempt to address this question.

Materials and methods

Patients with acute endothelial corneal graft rejection diagnosed at the Prince of Wales Hospital between April 1995 and December 1996 were recruited into this prospective study. Informed consent was obtained from all participating subjects and from parents of participating minors after full explanation of the nature, possible benefits and risks of the study.

Graft rejection was defined as an eye with a previously clear and thin graft, now showing some or all of the following signs: anterior chamber flare and cells, keratic precipitates on corneal endothelium, thickening of the graft, either diffusely or locally, and epithelial or endothelial rejection lines. Exclusion criteria included a refusal on the part of the patient, or the patient's guardian, to participate in the study, and the graft rejection having started more than 2 weeks (14 days) before presenting to us.

For those cases recruited into the study, the following treatment regimen was applied:

1. *Topical steroid*. One per cent prednisolone eyedrops (Pred Forte, Allergan) was given hourly, 24 h a day for 3 days, then hourly from 8 a.m. to 12 midnight for 4 days, and then tapered to four times a day after 2 weeks or earlier if the rejection episode showed signs of reversal.
2. *Pulsed methylprednisolone*. Intravenous infusion of 500 mg methylprednisolone over 1 h (prepared by dissolving 500 mg Solu-Medrone (methylprednisolone sodium succinate, Upjohn) in 100 ml 4% dextrose) was given once the diagnosis was made.
3. *Oral cyclosporin A*. Sandimmun Neoral (Sandoz) was used. The initial loading dose of 15 mg/kg per day was given for 2 days, followed by 7.5 mg/kg per day for a further 2 days. The maintenance dose was adjusted to achieve a therapeutic trough blood level of 100–200 μg/l and was continued for 6 months after the rejection was reversed.

As a measure of clinical response to the regime, the following parameters were monitored during the treatment period: the clinical signs on slit lamp examination, including the return of graft clarity and the normalisation of corneal thickness by ultrasound pachymetry, and the restoration of visual acuity, which was checked daily for 1 week. As a measure of the side effects of the regime, the following parameters were monitored during the treatment period: blood pressure, body weight, complete blood count (including lymphocyte count) daily from day 0 to day 3, renal function tests (serum urea and creatinine levels), blood cyclosporin A level, and the patient's symptomatology.

Results

A total of 11 patients were recruited into the study (Table 1). There were four male and seven female patients. Their ages ranged from 9 to 78 years old (mean age 54.8 years). In seven of these patients the rejected graft was their first graft. In the remaining four patients it was their second graft. Of these 11 patients one had had two previous rejection episodes and one had had one previous rejection episode. All the rest had their first graft rejection during the study period. The time from the corneal transplant operation to the episode of acute rejection ranged from 0.5 to 120 months (mean 24.7 months). The time delay from the onset of symptoms of rejection to presentation ranged from 1 to 14 days (mean 4.5 days).

Of these 11 cases, only one patient (case 5) who had no particular risk factors (first graft, no episode of previous graft rejection and presented on day 9) did not have the rejection reversed. All the other 10 cases had their rejection episode reversed, with a resultant clear graft. This translated into a 90.9% (10 of 11 eyes) success rate with this treatment regime for acute corneal graft rejection. The action of this regimen also appeared to be swift, with graft clarity re-established by 36–72 h after commencement of treatment in all 10 patients. The follow-up period, starting from the presentation of the graft rejection, ranged from 8 to 22 months (mean 16.5 months). No recurrence of rejection occurred during this follow-up period. The only side effect from the combined treatment in this group of patients was an endoscopically confirmed duodenal ulcer in one case. There were no other major side effects of treatment in any of the remaining patients. The duodenal ulceration healed completely after medical therapy.

Table 1. *Results of using combined pulse intravenous methylprednisolone and oral cyclosporin in the treatment of acute graft rejection*

Patient no.	Sex/Age (yr)	Surgery to rejection time (months)	Onset to treatment time (days)	Outcome	Follow-up time (months)	Side effects
1	F/48	18	2	Reversed	22	Nil
2	F/37	4	2	Reversed	21	Nil
3	F/75	10	7	Reversed	20	Nil
4	M/9	0.5	2	Reversed	19	Nil
5	M/70	3.5	9	**Rejected**	19	Nil
6	M/33	120	14	Reversed	18	Nil
7	F/65	15	2	Reversed	18	Nil
8	F/65	2.5	4	Reversed	13	Duodenal ulcer
9	M/60	33	5	Reversed	13	Nil
10	F/63	1.75	2	Reversed	11	Nil
11	F/78	63	1	Reversed	8	Nil
Mean	54.8	24.7	4.5	–	16.5	

Discussion

Oral cyclosporin A has been used primarily for prophylaxis against allograft rejection in high-risk patients.[11,12] Its main action is on the afferent limb of the cellular immune response, i.e. antigen presentation and lymphokine production, making its use before or shortly after new antigens (e.g. corneal allograft) are introduced both theoretically sound and clinically effective. Its role in acute graft rejection is less well defined. In 1984, Margreiter *et al.*[21] reported a prospective randomised trial in which 5 of 12 (41.7%) patients with acute rejection of their renal allografts had their steroid-resistant rejection episode reversed by supplementary oral cyclosporin A. Cyclosporin A may, therefore, also have a role in the reversal of acute graft rejection, apart from its prophylactic role. Cyclosporin A appeared to act synergistically with steroid in the reversal of these acute rejections. Our study here adds further supporting evidence that cyclosporin A may act as an adjunct alongside steroid in the treatment of acute corneal allograft rejection.

The exact mechanism of this adjunctive effect is not clear. However, cyclosporin A, while exerting its main effects on the afferent limb of immune response, may also possess some inhibitory influence on the efferent limb, e.g. by inactivating cytotoxic T lymphocytes.[11,19] This may explain its effectiveness in helping to reverse established acute rejections.[20,21]

Furthermore, cyclosporin A may also have an effect on the expression of interleukin-6 (IL-6). IL-6 is a pro-inflammatory cytokine produced during the acute phase of inflammation that can induce the differentiation of B and T lymphocytes, and the generation of cytotoxic T lymphocytes. On the one hand, it has been shown that the level of IL-6 in the transplanted lung correlates with the magnitude of the histological mononuclear cell infiltration of acute rejection.[22,23] On the other hand, its expression alters with the administration of cyclosporin A.[24] The exact mechanism of action of cyclosporin A in reversing established rejection is likely to be complex and to involve multiple pathways and factors. Much work has yet to be done to clarify these actions.

In our study, the success rate of reversing an established acute corneal graft rejection by the combined regimen of a single intravenous pulse of 500 mg methylprednisolone and oral cyclosporin A was 90.9%. This compares favourably with the 80% reported for single pulse intravenous methylprednisolone alone.[2] The low incidence and relative mildness of side effects were also encouraging. After successful reversal of the graft rejection, oral cyclosporin A acting as prophylaxis was continued for 6 months to lower the chance of recurrence of graft rejection. No recurrence of acute corneal graft rejection was encountered amongst the 10 patients in whom rejection had been successfully reversed by our regime during the study period.

An alternative to oral cyclosporin A is topical cyclosporin A. Topical cyclosporin A is expected to have fewer systemic side effects than oral cyclosporin A, the side effects of which include nephrotoxicity and blood pressure changes.[25] It would be interesting to see whether topical cyclosporin A, which has been reported to be effective as prophylaxis,[26,27] can replace oral cyclosporin A with a similar adjunctive effect in reversing established graft rejection.

Other immunosuppressive agents, such as FK-506, have also been tried as prophylaxis against allograft rejection in animal studies.[28,29] FK-506 is known for its high potency, which can be up to 10-100 times that of cyclosporin A. Its role in both the oral and topical forms to act as prophylaxis or as an agent to combat corneal graft rejection in human subjects is worth examining.

Although the results of this preliminary study are very encouraging, the sample size is too small to draw any definite conclusion. The quick response to the combined treatment is a major advantage as this may enable the survival of maximal numbers of endothelial cells. A larger-scale, double-masked, prospective study with a longer follow-up is recommended.

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