ORIGINAL ARTICLES—Clinical science

Mycophenolate mofetil versus cyclosporin A in high risk keratoplasty patients: a prospectively randomised clinical trial

Alexander Reis, Thomas Reinhard, Adina Voiculescu, Bernd Kutkuhn, Erhard Godehardt, Helga Spelsberg, Christoph Althaus, Rainer Sundmacher

Abstract

Background/aims—The requirement for an effective, minimally toxic immunosuppressive agent remains a major obstacle to performing high risk corneal transplantation. Although therapy with cyclosporin A (CSA) allows superior graft survival, its use is limited because of a wide range of side effects. Mycophenolate mofetil (MMF) has been shown to be a safe and effective immunosuppressive agent following renal transplantation. This prospective, randomised clinical trial was carried out to investigate the efficacy and safety of MMF in preventing corneal allograft rejection.

Methods—Recipients of corneal transplants who were at high risk for graft failure were randomly assigned to either CSA or MMF immunosuppressive therapy. CSA was given in doses to achieve whole blood trough levels of 120–150 ng/ml. MMF was given in a daily dose of 2 g. Both therapy groups additionally received oral corticosteroids (fluocortolone 1 mg/kg) which were tapered and discontinued within the first 3 postoperative weeks. Patients were monitored closely for evidence of corneal graft rejection and adverse side effects. Drug efficacy was measured, primarily, by the number of patients who experienced at least one episode of clinical graft rejection. Safety analysis focused on reported adverse side effects and laboratory measurements.

Results—41 patients were enrolled in the study. There was no statistically significant difference between the two groups. 20 patients received CSA and 21 patients received MMF. Two patients in each group showed evidence of acute graft rejection which could be treated effectively by corticosteroids. All corneal grafts remained clear throughout the follow up.

Conclusions—In this study it was shown that MMF is just as effective as CSA in preventing acute rejection following high risk corneal transplantation. Mycophenolate mofetil represents a promising alternative therapeutic option in patients who are at high risk for corneal graft failure.

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Eye Hospital, Heinrich-Heine-University, Duesseldorf, Germany
A Reis
T Reinhard
H Spelsberg
C Althaus
R Sundmacher

Department of Nephrology and Rheumatology
A Voiculescu
B Kutkuhn

Department of Biostatistics/ Cardiovascular Surgery
E Godehardt

Correspondence to:
Dr Alexander Reis, Eye Hospital, Heinrich-Heine University, Moorenstrasse 5, 40225 Duesseldorf, Germany

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The requirement for an effective, minimally toxic immunosuppressive regimen remains a major obstacle to performing high risk human corneal transplantation. Acute rejection is the major cause of up to 75% of transplant opacifications in some immunological high risk groups.[1][2] Cyclosporin A (CSA), a macrolide antibiotic which interferes with IL-2 transcription, is a very potent prophylactic agent for preventing corneal allograft rejection and is used in some specialised centres after high risk keratoplasty. Although therapy with CSA allows superior graft survival, its use is limited because of a wide range of side effects (for example, diabetogenicity, arterial hypertension, hyperlipidaemia, nephrotoxicity). In a retrospective study of the safety and effectiveness of CSA at this centre, 10% of patients taking CSA suffered from side effects.[1] Additionally, for CSA to be effective, the daily dose should be adjusted to keep blood levels between 120–150 ng/ml, which leads to very costly, labour intensive laboratory drug monitoring.

Mycophenolate mofetil (MMF, the morpholinoethylester of mycophenolic acid) is the first immunosuppressant in the past 10 years that has been approved for clinical use by the Food and Drug Administration in the United States for preventing allograft rejection following renal transplantation. Its safety and effectiveness in combination with CSA following kidney transplantation has already been proved in several clinical studies.[3-5] Unlike CSA, mycophenolic acid does not interfere with IL-2 pathways. Mycophenolic acid reversibly inhibits the de novo formation of guanosine nucleosides[6] by inhibiting the enzyme inosine monophosphate dehydrogenase. As T and B cells are predominantly dependent on the de novo synthesis of guanosine nucleosides, the purine biosynthesis of these cells is relatively selectively inhibited.[7] The potency of this drug and its synergistic effect with CSA in delaying corneal allograft rejection has already been proved in the rat keratoplasty model.[8]

This prospective, randomised clinical trial was carried out to investigate the efficacy and safety of MMF as an immunosuppressive agent compared with CSA in preventing corneal allograft rejection after high risk corneal transplantation.

Table 1 Characteristics of corneal graft recipients

	CSA	MMF
Mean (range) age (years)	58.7 (23.0–87.0)	57.5 (23.1–87.2)
Male	7 (35%)	9 (42.9%)
Female	13 (65%)	12 (57.1%)
Follow up (range) (months)	11.6 (3.6–20.5)	10.1 (4.4–21.5)
Original corneal disease		
Graft failure after corneal transplant because of		
Bullous keratopathy	5 (25%)	7 (33.3%)
Keratoconus	1 (5%)	4 (19.0%)
Granular dystrophy	2 (10%)	1 (4.8%)
Bacterial corneal ulcer	1 (5%)	0 (0%)
Perforating corneal injury	1 (5%)	1 (4.8%)
Total of secondary transplants	10 (50%)	14 (66.7%)
Keratokonus, severe atopic dermatitis	1 (5%)	3 (14.3%)
Scarring and neovascularisation post keratitis	3 (15%)	1 (4.8%)
Granular dystrophy (limbokeratoplasty)	2 (10%)	0 (0%)
Eccentric keratoconus (requiring 9.2 mm graft)	0 (0%)	2 (9.5%)
Bullous keratopathy, glaucoma (steroid responsive)	4 (20%)	2 (9.5%)

Methods

PATIENT SELECTION

Only allogeneic, orthotopic corneal transplant recipients at high risk of graft failure because of immunological corneal graft rejection were considered eligible for the study. High risk is defined as having three or four quadrants of corneal neovascularisation, a history of previous corneal graft rejection, transplantation of a highly immunogenic graft (limbokeratoplasty), severe atopic dermatitis, steroid glaucoma, and positioning of the graft close to the limbus (Table 1). Randomisation into study groups was done at the time of joining the waiting list for the operative procedure by drawing lots. At this time a complete medical history and physical examination were performed for each patient with particular attention given to any malignancy, hepatic or renal disease, and gastrointestinal disorders.

The protocol excluded patients with a history of malignant disorders, serological evidence of HIV or HbsAg, systemic infections that required therapy at the time of entry, active peptic ulcer disease, inadequate contraceptive measures, pregnancy, or patients aged 18 years and younger. Patients with a history of herpetic eye disease or acute corneal infection were also excluded from this study.

Formal approval from the ethics committee of the Heinrich-Heine-University and a written informed consent were obtained for each patient enrolled. The study was carried out according to the Declaration of Helsinki.

Enrolled patients were equally and randomly assigned to one of the two treatment groups, CSA or MMF. The dose of CSA (Sandimmun optoral, Sandoz) was adjusted to the whole blood trough levels with a target range of 120–150 ng/ml (monoclonal TDx, Abbott Exsym).

Table 2 Characteristics of donors and grafts

	CSA	MMF
Mean (range) donor age (years)	60.5 (32.0–84.0)	60.15 (24.0–79.0)
Mean (range) time from donor death to graft explant		
(hours)	17.5 (1.0–72.0)	12.0 (1.0–37.0)
Mean (range) culture time (days)	17.1 (10.0–23.0)	16.35 (10.0–21.0)
MHC class I match		
A	0	0
B	0	0
C	1 (5%)	0
MHC class II match		
DR	2 (10%)	1 (4.8%)
DQ	3 (15%)	2 (9.5%)

During the early postoperative period, blood CSA levels and blood chemistry were estimated twice weekly. With stabilisation of the CSA levels the frequency of the tests was reduced. MMF was given in a dosage of 2 g (1 g twice daily). Routine blood samples were drawn every 2–4 weeks to check for drug toxicity. Both therapy groups additionally received topical and systemic corticosteroids according to the routine practice of our clinic—that is, systemic fluocortolone (Ultralan, Schering Health) 1 mg/kg body weight, tapered during the first 3 postoperative weeks and stopped thereafter and topical prednisolone acetate 1% (Inflanefran forte, Pharm-Allergan, Germany) five times a day, tapered during the first 5 postoperative months. In patients with steroid induced glaucoma topical steroids were tapered off within 1 week. Both CSA and MMF were administered for the first 6 postoperative months and then tapered off within 2 weeks.

The donor grafts were mostly 7.7 mm in diameter and 0.2 mm larger than the recipient bed. Donor corneas were preserved as corneoscleral discs in organ culture medium (minimal essential medium, antibiotics, antimycotics) at 34°C. Endothelial cell density before surgery was at least 2100 cells/mm. The keratoplasty technique was identical in all patients. A double running cross stitch nylon suture (Ethibond 10.0, Ethicon, Germany) was used, and when necessary simultaneous cataract surgery was performed. We undertook serological tissue typing of major histocompatibility class I antigens (A, B, C) and DNA tissue typing of MHC class II antigens (DR, DQ) of both the donor and recipient. No active matching for donor and recipient MHC antigens were performed.

Patients were closely monitored for evidence of graft rejection and for adverse side effects such as hepatotoxicity, nephrotoxicity, neurotoxicity, and gastroenterotoxicity. Patient follow ups were done at 1, 3, 6, 9 and 12 months after transplantation during which laboratory assessments were performed. General practitioners also followed these patients between their regular monthly intervals monitoring white blood cell count, serum biochemistry, and CSA trough levels.

Efficacy of the treatment groups was defined by the number of patients who experienced at least one episode of a clinically manifested graft rejection (corneal precipitates, Khoudadoust line, stromal oedema) or a premature withdrawal from the study for any reason. The treatment of rejection consisted of hourly prednisolone acetate 1% drops. In severe cases, oral fluocortolone 1 mg/kg was added and tapered according to the clinical situation.

Safety analysis focused on spontaneously reported adverse side effects and irregular laboratory measurements.

STATISTICAL ANALYSIS

Treatment groups were compared using a two way analysis of variance (ANOVA). The difference between males and females was assessed with the χ^2 test for independence and unequal variances. Time to first rejection was calculated

Table 3 Rejection, treatment failure, adverse events

	CSA	MMF
Rejection*		
In the first 6 postoperative months	1 (5%)	1 (4.5%)
After cessation of immunosuppressive therapy	1 (5%)	1 (4.5%)
Irreversible graft failure from rejection	0	0
Premature withdrawal	1 (5%)	2 (9.1%)
Hepatotoxicity	1 (5%)	0
Hodgkin's lymphoma	0	1 (4.5%)
Unsatisfactory therapeutic response	0	1 (4.5%)

*The rejection episodes involved different grafts, no graft was rejected twice.

as the time to the event from the date of transplant and evaluated with the Kaplan–Meier estimator. For all tests a p value less than 0.05 was considered statistically significant.

Results

DEMOGRAPHY
A total of 41 patients (16 men, 25 women, aged 23–87 years) were enrolled in the study; 20 patients were assigned to receive CSA and 21 patients were assigned to receive MMF. The patient demographics broken down to each treatment group are shown in Table 1. There was no significant difference between the two groups when comparing the average donor age at the time of transplantation, recipient age, time from donor death to graft explantation, corneal culture time, graft endothelial cell count, and human histocompatibility leucocyte antigen matching (HLA) (Tables 1 and 2). There were statistically significantly more female (61.0%) than male patients (39.0%) enrolled in the study. However the ratio of male/female was not statistically significantly different between the two study groups.

EFFICACY OF MMF *v* CSA
The incidence of graft rejection is shown in Table 3, and a Kaplan–Meier survival plot in Figure 1. There was one patient who showed acute graft rejection despite immunosuppressive therapy both in the CSA (2nd postoperative month) and in the MMF (6th postoperative month) group. Likewise one patient in each group suffered an acute rejection episode 8 months and 12 months after cessation of sys-
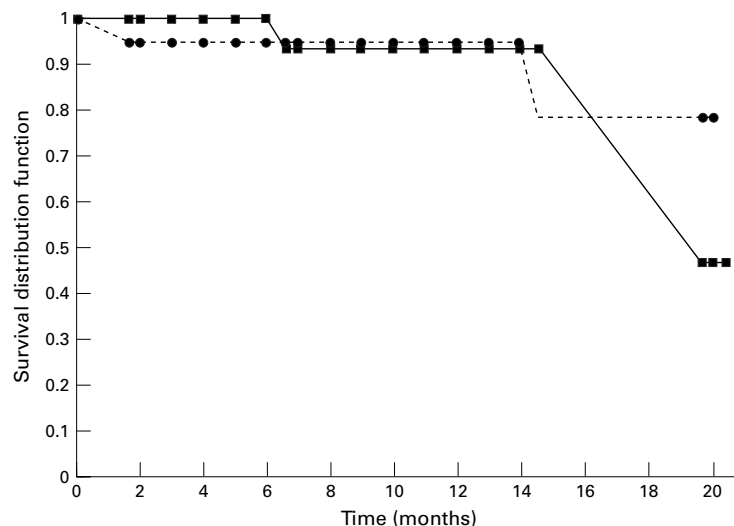
temic immunosuppression in both the CSA and MMF group respectively. This leads to an overall rejection rate of 10% in the CSA and 9.5% in the MMF group. These rejection episodes were treated effectively with both systemic and topical steroids. All corneal grafts of both the CSA and MMF groups remained clear throughout the follow up period.

ADVERSE SIDE EFFECTS
In the CSA group, one patient experienced hepatotoxicity (elevated γ-GT) that resulted in premature discontinuation of the study drug 5 months after transplantation.

In the MMF group one patient had to discontinue MMF because a Hodgkin's lymphoma was diagnosed 1 month after keratoplasty. In a second MMF patient with severe atopic dermatitis MMF was substituted by CSA 2 months after transplantation, not for adverse side effects but because there was far too little improvement of the dermatological disease with MMF. This patient's skin improved after the switch to CSA. No opportunistic infections, gastrointestinal disorders, leucopenia, or anaemia were diagnosed in either of the CSA and MMF groups. The incidence of adverse side effects was not statistically significantly different between the two treatment groups.

Discussion
The widespread acceptance of corneal transplantation as the treatment of choice for most patients with blinding corneal disease can, for the most part, be attributed to the absence of antigen presenting cells in the donor tissue, the avascularity of the recipient site and, to some extent, to the anterior chamber associated immunodeviation (ACAID).[9][10] Because of this immune privilege corneal allografts remain clear without any supplemental immunosuppressive regimen in up to 95% of transplant patients within 5 years after transplantation in those who are considered at "normal" risk for immunological graft rejection.[1] However, patients who are at high risk for corneal graft rejection experience an opacification of the allograft in up to 75%.[1][2] The criteria for high risk are not yet standardised. The most widely accepted definitions are corneal neovascularisation involving three or four quadrants, a history of previous transplants, and an extra large corneal graft which comes into close proximity to the limbus. In this trial, patients with severe atopic dermatitis, patients with steroid responsive glaucoma, and patients who received a highly immunogenic limbokeratoplasty were also included. These conditions have been shown to be deleterious for corneal allografts in a retrospective analysis.[11] Based on these data we generally do not perform high risk keratoplasty without postoperative immunosuppression. Although the incidence of postoperative graft failure following high risk keratoplasties in our clinic dropped from 28% to 0% within a follow up of 3 years when using CSA,[1] this therapeutic regimen comes with a high range of side effects and a cost intensive follow up.

Figure 1 Kaplan–Meier survival plot. (●) CSA; (■) MMF.

MMF has been shown to be an effective immunosuppressive agent with a wide therapeutic range following renal transplantation. This study was designed to evaluate the efficacy and safety of MMF for the prevention of corneal allograft rejection. Acute rejection was chosen as a target criterion for the evaluation of the efficacy of immunosuppressive drugs because of the limited ability of 1 year graft survival to detect immunological effects.

MMF was found to be equally effective in preventing corneal allograft rejection compared with CSA. All grafts remained clear within the follow up period of approximately 1 year. Both drugs have been found to significantly reduce the incidence of rejection when compared with historical control groups.[1] In this study, there was one premature withdrawal in the CSA group and two withdrawals from the MMF group. Whereas the rising blood level of γ-GT in the CSA group can clearly be attributed to CSA, the two withdrawals in the MMF group may not be as easily related to the study drug. One patient was switched from MMF to CSA because of lack of improvement of her atopic dermatitis during immunosuppression. This decision was based on previous clinical experiences with systemic CSA in improving atopic dermatitis and therefore may not be considered as a clear treatment failure. The second study withdrawal from the MMF group was due to the diagnosis of Hodgkin's lymphoma as early as 1 month after the transplantation. As MMF is not an alkylating agent and does not cause DNA miscoding, the manifestation of this disease may be attributed any general systemic immunosuppression. The very short interval between initiation of therapy and detection of the lymphoma also favours a random correlation of both events.

Interestingly, no gastrointestinal side effects have been seen in the MMF group. These adverse events were evident in up to 45% of patients following renal transplantation[4] and may be explained with the generally worse medical condition of renal graft recipients compared with corneal graft recipients.

The need for blood level adapted dosing for MMF remains controversial. All data concerning efficacy and safety of this drug are from clinical trials with a fixed daily dose of 2 g or 3 g. Therefore we have chosen a dose of 2 g MMF per day and feel that blood level measurements should be reserved to special situations (for example, paediatric patients, treatment failure, adverse events). The broad therapeutic range makes MMF especially appealing for ophthalmic patients who generally do not visit their ophthalmologist as often as renal transplant recipients would visit their nephrologist. It is also worth noting that the cost and logistics of postoperative immunosuppression are reduced as a result of the omission of monitoring drug titres.

Although HLA (MHC) typing were performed for class I and class II antigens for both the donor and recipient, donors and recipients were not matched with regard to their MHC antigens. Despite the transplantation of mismatched grafts in both groups we found no graft loss within the follow up period.

In this study we have shown for the first time clinically that MMF in combination with a short postoperative course of oral steroids is just as effective as CSA and oral steroids in preventing acute rejection following high risk corneal transplantation. In fact the efficacy and safety of this new compound in preventing corneal allograft rejection was comparable with CSA with 11 months of follow up. Mycophenolate mofetil represents a promising alternative therapeutic option in patients who are at high risk for corneal graft failure and should therefore be further investigated in controlled long term studies. The wide therapeutic range and the omission of drug monitoring makes this compound especially interesting for ophthalmic patients.

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