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Systemic mycophenolate mofetil in comparison with systemic cyclosporin A in high-risk keratoplasty patients: 3 years' results of a randomized prospective clinical trial

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Abstract *Background:* With the use of systemic cyclosporin A (CsA), graft prognosis after high-risk penetrating keratoplasty has improved considerably. However, the application of CsA is limited owing to a variety of severe side effects. In this prospectively randomized study mycophenolate mofetil (MMF), a safe and efficient immunosuppressive medication after renal transplantation, was compared with CsA after high-risk penetrating keratoplasty. *Methods:* Twenty-nine high-risk keratoplasty patients were treated with MMF 2× 1 g daily; another 27 patients received CsA, aiming at blood trough levels of 120–150 ng/ml. Systemic immunosuppression was scheduled for 6 months. In both groups oral corticosteroids (fluocortolone 1 mg/kg) were administered for 3 weeks postoperatively. *Results:* During the first year after operation, no graft failure was recorded. Two years postoperatively 92%/82% and 3 years postoperatively 74%/69% of grafts were clear in the MMF and CsA group, respectively (Kaplan Meier P=0.33, log-rank test). In total, two graft failures were recorded in the MMF group and four in the CsA group. Three years postoperatively 53% of the grafts were rejection-free in the MMF group and 73% in the CsA group (Kaplan Meier P=0.46, log-rank test). Eight endothelial immune reactions were observed in the MMF group (three under systemic immunosuppression/five thereafter; six mild/two severe) and five in the CsA group (three under systemic immunosuppression/two thereafter; three mild/two severe). Side effects occurred in six patients under MMF and 11 under CsA. *Conclusions:* Concerning efficacy, no statistically significant difference between systemic MMF and systemic CsA administered for 6 months after high-risk penetrating keratoplasty could be shown. Systemic MMF was proven to be at least as safe as CsA. The main mechanism in improving graft survival is a shift from severe to milder endothelial immune reactions, as already demonstrated for CsA. Thus, MMF may become an alternative to CsA for immunosuppression after penetrating high-risk keratoplasty. About 2 years postoperatively, pharmacologically induced relative immunological tolerance slowly decreases. Therefore, long-term administration of systemic MMF should be evaluated in further studies.

Introduction

Short-term administration of systemic cyclosporin A (CsA) has led to considerable improvement in graft prognosis for high-risk penetrating keratoplasty patients [7, 8, 14]. However, side effects such as nephrotoxicity, hepatotoxicity and arterial hypertension occur in about 10% of patients treated with CsA as systemic monoimmunomodulation for 6–12 months postoperatively [14]. In order to minimize the risk of side effects, CsA

blood trough levels should not exceed 150 ng/ml. Therefore, expensive laboratory monitoring is necessary.

Mycophenolate mofetil (MMF), the morpholinoethylester of mycophenolenic acid (MPA), is a potent inhibitor of inosine monophosphate dehydrogenase. MPA inhibits the proliferation of T and B lymphocytes [12]. The efficacy and safety of MMF in a daily dose of 2× 1 g combined with CsA and corticosteroids has been shown after kidney transplantation [4, 5, 24].The efficacy of MMF after penetrating keratoplasty has been demonstrated in the rat model [16]. In a prospective randomized study, short-term results after mean follow-up of about 10 months showed MMF to be an effective and safe potential alternative to CsA for therapy after high-risk penetrating keratoplasty [17]. Now, efficacy and safety data for a larger group of patients with a much longer follow-up of up to 3 years can be presented.

Patients and methods

Patient selection, treatment and follow-up

Fifty-six patients undergoing high-risk penetrating keratoplasty were enrolled in this single-center study. All patients gave written informed consent after obtaining approval by the local ethics committee. The study was performed in accordance with the Declaration of Helsinki. High risk was defined by the presence of deep vascularization in three or four quadrants, a history of previous keratoplasty, position of the graft close to the limbus, transplantation of a highly immunogenic graft (limbokeratoplasty), severe atopic dermatitis or steroid response glaucoma (Table 1). In eight keratoconus patients with rekeratoplasty (three in the CsA, five in the MMF group) first keratoplasty had been performed elsewhere. Rekeratoplasty was judged necessary because of endothelial failure (two patients in the CsA, two in the MMF group), scarring of the graft due to atopic blepharokeratoconjunctivitis (no patients in the CsA, two in the MMF group), and for refractive reasons (one patient in the CsA, one in the MMF group). In six patients with granular and lattice dystrophy rekeratoplasty was performed because of recurrence of the underlying dystrophies. In all six of these patients superficial keratectomy had been performed had at least once prior to rekeratoplasty, and in all six patients repeat keratoplasty took the form of limbokeratoplasty. Steroid glaucoma in combination with bullous keratopathy was regarded a high-risk situation since no topical steroids can be applied postoperatively – a steep increase in the incidence of immunological events in such a situation is known [22, 23].

Patients with a history of malignant tumors and those with acute or chronic systemic infections, acute peptic ulcer disease, pregnancy or insufficient contraceptive measures were excluded, as were those under 18 years of age. Herpetic eye disease or any other kind of acute corneal infection also led to exclusion. All patients underwent physical and laboratory examination prior to surgery.

The 56 patients were randomized to receive CsA (27 patients) or MMF (29 patients). MMF was administered in a daily dose of 2× 1 g; the CsA dose was adjusted according to blood trough levels, with a target of 120–150 ng/ml (monoclonal TDx, Abbott Exsym). After 6 months, immunosuppressive medication was tapered within 2 weeks. Additionally, all patients except those with steroid-induced glaucoma received corticosteroids systemically (1 mg/kg body weight fluocortolone, tapered within 3 weeks postoperatively) and topically (5 drops prednisolone acetate 1% daily after epithelial consolidation, tapered within 5 months).

Postoperatively, all patients were monitored for efficacy (clear graft survival, immune reactions, endothelial cell loss) and drug side effects (hepato-, nephro-, neuro- and gastroenterotoxicity). Postoperative visits were scheduled after 1, 3, 6, 9, 12, 24 and 36 months. At every visit, the patients underwent evaluation of visual acuity, slit-lamp examination, specular microscopy of the graft, estimation of intraocular pressure and examination of the retina. Furthermore, physical examination and blood tests were carried out. Between attendances the patients were closely examined by their general practitioners.

Surgery was performed using grafts preserved in organ culture with an endothelial cell density of at least 2000 cells/mm^2 (Table 2). The HLA types of donor and recipient could be determined in 15 cases in the CsA group and in 18 cases in the MMF group; in the remaining cases HLA typing of the donor failed (Table 2). HLA typing was performed using serological tests for class I and PCR for class II. There was no statistically significant difference between the two groups concerning distribution of HLA mismatches (χ^2 test, P=0.59). A 10.0 nylon double-running cross-stitch suture was used for graft fixation, and, if necessary cataract

Table 1 Data of graft recipients

	CsA	MMF	Stat. test	(P)
1. Patients	27	29		
2. Male/female	13/14	14/15		
3. Mean age (years)	57.7 (28–87)	52.5 (18–86)	*t*-test	(0.30)
4. Follow-up (months)	19.3 (7–36)	19.8 (6–36)	*t*-test	(0.80)
5. High risk factor			χ^2 test	(0.80)
a) Graft failure after previous keratoplasty	15	19		
Keratokonus	3	5		
Granular/lattice dystrophy	3	3		
Scars	3	4		
Bullous keratopathy	3	5		
Fuchs' dystrophy	2	2		
Bacterial ulcer	1	0		
b) Steroid induced glaucoma (bullous keratopathy)	4	2		
c) Severe atopic dermatitis (keratokonus/keratitis)	2	3		
d) Limbo-keratoplasty (granular/lattice dystrophy)	3	1		
e) Deep vascularization in 3/4 quadrants (scars)	3	2		
f) Graft position close to limbus (eccentric keratokonus)	0	2		

Table 2 Graft and HLA data

	CsA	MMF	Stat. test	(*P*)
1. Donor age (years)	61.1 (32–84)	55.1 (19–87)	*t*-test	(0.19)
2. Time death/graft preparation (hours)	17.7 (0.5–72.0)	11.5 (0.5–43.5)	*t*-test	(0.17)
3. Organ culture period (days)	17.3 (10–32)	17.0 (12–27)	*t*-test	(0.72)
4. Endothelial cell density, preoperative (cells/mm^2)	2400 (2088–3000)	2410 (2050–3060)	*t*-test	(0.72)
5. Graft diameter				
7.7 mm (*n*)	22	21	χ^2 test	(0.32)
8.2–9.7 mm (*n*)	5	8		
6. Triple procedures/penetrating keratoplasty	4/23	5/24	χ^2 test	(0.54)
7. HLA mismatches				
0	2	0		
1	2	2		
2	0	1		
3	1	2		
4	4	3		
5	4	5		
6	2	5		
Donor HLA-untyped	12	11	χ^2 test	(0.59)

surgery was performed simultaneously. Postoperatively, immune reactions were diagnosed by endothelial precipitates adhering to graft endothelium with (severe endothelial immune reactions) or without (mild endothelial immune reactions) stromal edema or by the presence of non-infectious stromal infiltration (stromal immune reactions) [1, 13, 15, 19]. These patients were treated with prednisolone acetate 1% eye drops hourly and, in severe cases, additionally with 1 mg/kg body weight fluocortolone.

Endothelial cell loss was assessed only in patients with at least three postoperative endothelial cell density values. Patients with endothelial immune reactions were excluded from this calculation. The individual mean loss of endothelial cells per day and per square millimeter was derived from the postoperatively acquired endothelial values of each patient individually. This was done by calculating the slope of the regression line for each scatter plot of endothelial cell density values plotted against time [2, 11].

Statistical methods

Treatment groups were compared using the *t*-test for independent samples (patient age, follow-up, donor age, period between donor's death and graft preparation, organ culture period, preoperative and postoperative endothelial cell density) or the χ^2 test (indication for surgery, graft diameter, HLA data, distribution of side effects). Efficacy parameters were clear graft survival and occurrence of clinically detectable immune reactions. Calculation was done using the Kaplan-Meier estimator, evaluation for statistical significance by means of the log-rank test. A *P* value below 0.05 was considered to show a statistically significant difference.

Results

Demography

There was no statistically significant difference between the 2 treatment groups concerning patient age, follow-up, indication for surgery, donor age, period between donor's death and graft preparation, organ culture period, preoperative endothelial cell density, graft diameter and HLA data (Tables 1, 2).

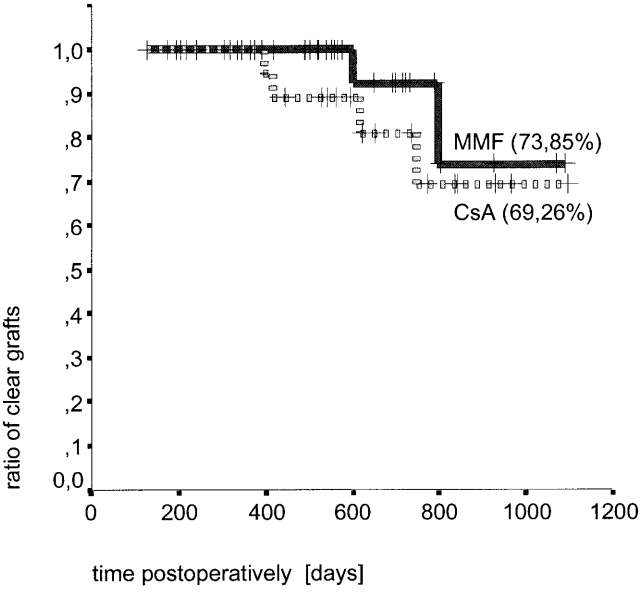

Fig. 1 Kaplan-Meier clear graft survival curves (log-rank test: *P*=0.33)

Efficacy

In both groups, no graft loss was observed during the first postoperative year. During the whole follow-up, four grafts failed in the CsA group and two grafts in the MMF group (Table 3). Three years postoperatively, 69% of the grafts were clear in the CsA group and 74% in the MMF group. Graft survival did not show a statistically significant difference between the treatment groups (Fig. 1). Reasons for graft failure were glaucoma (2 cases in the CsA group, 1 in the MMF group), graft infection (1 in the CsA group) and endothelial immune reactions (1 in the CsA group, 1 in the MMF group).

Five patients in the CsA group and eight in the MMF group experienced endothelial immune reactions (Table 3). In both groups the majority of these reactions were mild. Only one graft (in the CsA group during immunosuppressive therapy) was rejected twice. Three years postoperatively, 73% of the grafts in the CsA group and 53% of those in the MMF group were free of immune reactions. However, this difference was not statistically significant (Fig. 2). The respective error of the second kind (beta) was 0.42 for the sample sizes tested. In both treatment groups most immune reactions were reversible (4/5 in the CsA, 7/8 in the MMF group). Neither the two CsA patients without HLA mismatches nor the two CsA patients with six HLA mismatches experienced immune reactions or graft failure. In the MMF group, one of the five patients with six HLA mismatches experienced two mild, reversible endothelial immune reactions, another one graft failure due to glaucoma decompensation. Concerning endothelial cell loss, no statistically significant difference between the two groups was found (Table 3).

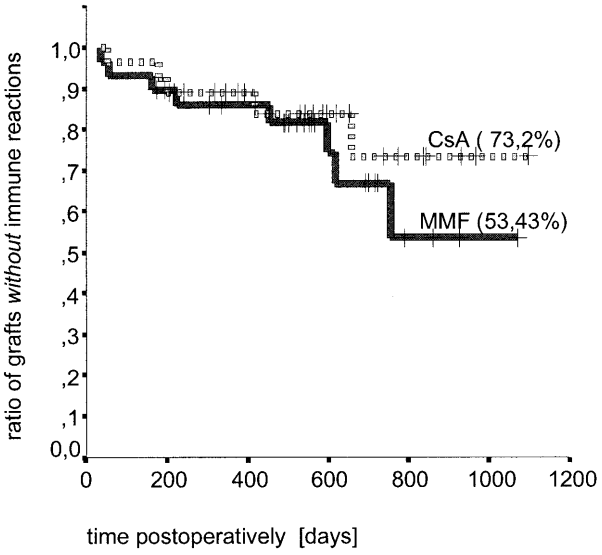

Fig. 2 Absence of immune reactions calculated according to Kaplan-Meier (log-rank test: P=0.46)

Safety

In the CsA group, premature withdrawal of immunosuppressive prophylaxis was performed in two patients (one case of severe gingiva hyperplasia, one of hepatotoxicity), and in the MMF group in further two patients (one diagnosis of Hodgkin's lymphoma 1 month after keratoplasty, one of dermatological problems in a patient with severe atopic dermatitis). Side effects were observed in 11 patients in the CsA group and 6 patients in the MMF group (Table 3).

Table 3 Efficacy and safety data

	CsA patients (*n*)	MMF patients (*n*)	Stat. test
1. Graft failure (*n*)			
During/after immunosuppression	0/4	0/2	
2. Reasons for graft failure (*n*)			
Immune reaction	1	1	
Glaucoma	2	1	
Graft infection	1	0	
3. Immune reactions (*n*)			
During immunosuppression			
Mild	2	2	
Severe	1[a]	1	
After immunosuppression			
Mild	1	4	
Severe	1	1	
4. Endothelial cell loss (loss of cells/day × mm^2)	–2.03 (–6.8 to 0)	–1.26 (–4.6 to +0.5)	*t*-test (0.25)
5. Side effects			χ^2 test (0.12)
Hepatotoxicity	3[b]	2	
Arterial hypertension	3	0	
Gingiva problems	3[b]	0	
Neurovegetative disorders	3	0	
Hodgkin's lymphoma (1 month after keratoplasty)	0	1[b]	
Recurrence of acoustic neurinoma (7 months after keratoplasty)	0	1	
Exacerbation of atopic dermatitis	0	1[b]	
6. Premature withdrawal of drug	2	2	

[a] Two severe immune reactions in the same patient under CsA
[b] One patient out of each group with premature withdrawal of the drug

Discussion

The avascularity of the recipient cornea, reduced expression of MHC class I molecules, the absence of MHC class II molecules, the secretion of immunomodulatory molecules by the cornea itself, corneal expression of complement-inhibiting molecules and the immune privilege (anterior chamber associated immune deviation, ACAID) of the anterior chamber are responsible for the excellent prognosis of corneal grafts in "normal"-risk keratoplasty patients [21]. Without systemic immunosuppression more than 90% of HLA-untyped grafts remain clear in the long run in such a situation [6, 18, 20, 25]. In high-risk keratoplasty, i.e. in patients with deep host cornea vascularization in three or four quadrants, a history of previous keratoplasty, position of the graft close to the limbus or transplantation of a highly immunogenic graft (limbokeratoplasty), up to 75% of high-risk grafts fail due to immune reactions [7, 8, 22].

The benefit of systemic CsA in reducing immune reactions is unequivocal in organ transplantation [10]. The effectiveness of systemic CsA in corneal high-risk transplantation has been proven in several experimental [3,9] and clinical [7, 8,14] studies. However, severe side effects are responsible for premature withdrawal of the drug in about 10% of the patients [14]. Therefore, there is a need for immunomodulatory alternatives. In this study, MMF is the first immunosuppressant since the introduction of systemic CsA into corneal surgery 15 years ago being clinically investigated in a prospective randomized trial after penetrating keratoplasty.

Concerning efficacy, i.e. clear graft survival, incidence of immune reactions and endothelial cell loss, no statistically significant difference was found between systemic MMF and systemic CsA administered for 6 months after high-risk penetrating keratoplasty. This may indicate that systemic MMF is at least as effective as systemic CsA. However, the respective error of the second kind (beta) was only 0.42 for the log rank test of Fig. 2 (ratio of grafts *without* immune reactions), i.e., with a probability of 0.58 (power of the test) a real difference between the treatment effects would have been detected using the sample sizes in this study. According to our findings, to arrive at the conclusion that the 2 treatment procedures are statistically equivalent, the power of the test would have to be increased to 0.95, i.e., 250 patients per group would have to be treated with a non-significant difference in outcome. This high number indicates that there is a clinically negligible difference between the two treatment effects. There is currently no statistical procedure to test the equivalence of two survival curves.

Eight patients experienced immune reactions during or after treatment with MMF. In these patients, the novel immunosuppressant was insufficient to induce complete immune tolerance. However, six of these eight immune reactions were mild and could be reversed, as could one of the two severe immune reactions. This shift from severe to milder endothelial immune reactions has already been demonstrated as the main mechanism by which CsA improves graft survival [14] and now seems to be true as well for MMF. Probably, in those patients with mild immune reactions only some incomplete immune tolerance has been induced. The first graft failure in the MMF group was noted 2 years postoperatively. One may conclude that, at least in some eyes, pharmacologically induced immune tolerance decreases after that period of time. Here, the question arises of whether long-term administration of systemic immunosuppressives is justified to maintain immune tolerance and improve graft survival. In light of the similar efficacy of the 2 drugs tested in this study and the higher risk of severe side effects in the CsA group, MMF seems to be more likely to be used for extension of the treatment period. Furthermore, in contrast to CsA, blood level-adapted dosing of MMF seems necessary only in special situations (e.g. treatment failure, severe side effects), resulting in lower costs for drug monitoring. Finally, the broad therapeutic range makes MMF more likely to be administered in patients with suboptimal compliance who fail to visit the ophthalmologist or general practitioner on a regular basis.

Side effects were observed in only 6 of 29 patients in the MMF group, in contrast to 11 of 27 patients in the CsA group. The main side effects in the MMF group were neurovegetative disorders, e.g., tremor and vertigo. These side effects were well tolerated by most of the patients and vanished completely after cessation of immunosuppressive therapy. Hodgkin's lymphoma was detected 1 month after keratoplasty in one patient. Given the short period between keratoplasty and diagnosis of the lymphoma, occurrence of the latter may have been coincidental. Probably, the same is true for the case of recurrence of acoustic neurinoma 7 months after keratoplasty.

In summary, no statistically significant difference in efficacy between systemic MMF and systemic CsA administered for 6 months after high-risk penetrating keratoplasty could be shown. Systemic MMF was proven to be at least as safe as CsA. Pharmacologically induced tolerance, however, decreases in the long run. Therefore, long-term administration of MMF after high-risk penetrating keratoplasty should be investigated.

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