Penetrating keratoplasty (PKP) has emerged as the most common form of solid tissue transplantation over the past 30 years. This relative success of PKP is attributable to continued advances in surgical techniques, equipment, ocular pharmacology and immunology, corneal storage, and eye banking procedures.

For uncomplicated first grafts performed in avascular «low-risk» beds with only local immune suppression, success rate is as high as 90%. This success in low-risk corneal transplantation, however, is overshadowed by the results of corneal grafts placed in «high risk» beds with rejection rates approaching 70%, even with maximal local and systemic immune suppression. So, although the cornea is classically described as possessing immunological privilege, immunologic corneal graft rejection is still the leading cause of graft failure after penetrating high-risk keratoplasty. In vascularised corneas and possibly corneas that have previously rejected a graft, the «immunological privilege» breaks down and the cornea becomes as susceptible as any other vascularised tissue in the body to rejection. Although much is being learned in laboratory science about this problem, not much has changed from a clinical standpoint and corneal grafting in high-risk corneas remains a significant challenge.

In 1949, when cortisone became available, it was used to prevent graft rejection improving prognosis in high-risk corneas, but it is since the introduction of short-term systemic immunosuppression with cyclosporine A (CsA) in the mid-1980s that graft prognosis in such cases has improved considerably. However, the use of CsA is limited because its side effects, especially nephrotoxicity and hepatotoxicity, alterations in glucose metabolism, hypertension, and gingival hyperplasia, which occurred in up to 41% of the cases, were significant challenges.

Purpose: Graft prognosis after penetrating high-risk keratoplasty has improved considerably with the use of systemic immunosuppressive medications. In this clinical study, we analyzed the efficacy and safety of Mycophenolate mofetil (MMF) in preventing corneal graft rejection.

Methods: A total of 99 keratoplasties were analyzed prospectively along 32 months: 59 cases received systemic immunosuppression with Mycophenolate mofetil (MMF) at a daily dose of 2 g, scheduled for 12 to 24 months. A control group of 40 cases did not received systemic immunosuppression. Every patient was treated with topical antibiotics (ciprofloxacin) and topical corticosteroids (prednisone). Metrical data of treated and non-treated groups are compared using chi-square test and logistic-regression analysis.

Results: The percentage of graft rejection was 22,5% in the non MMF group, and 11,86% in the MMF group. The MMF-treated patients showed few side effects and they were reversible. Logistic regression analysis revealed that MMF reduces the probability of graft rejection at 77,72% in any level of risk (adjusted odds ratio 0,22, \( P = 0.02280 \)).

Conclusion: We found a significant effect of MMF in preventing immune reaction, mainly after high-risk keratoplasty. Despite a shorter MMF administration compared with other systemic immunosuppressants like cyclosporine A (CsA), it was shown to have comparable potency regarding clear graft survival and good tolerancy.

Keywords: Immunosuppression, Mycophenolate mofetil, rejection, keratoplasty.

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patients. The need for less toxic approaches to prevent and treat immune rejection is therefore apparent and has led to numerous new approaches.

Mycophenolate mofetil (MMF) has shown its efficacy and safety after kidney transplantation (in combination with CsA and corticosteroids) and after heart and liver transplantation. In 1997 was administered for the first time as postoperative treatment after penetrating high-risk keratoplasty and patients with low-risk keratoplasties that wanted to be treated. The risk of graft rejection was defined on this pathway than other cell types are. MPA suppresses DNA synthesis and proliferation of T lymphocytes. So, MMF inhibits the proliferation of human T and B lymphocytes, the proliferation of these cells is selectively inhibited. The development of MMF was the first application of human genetics to define a therapeutic target. The substance is administered at a fixed dose of 2x1 g per day with few side effects, mainly gastrointestinal disturbances caused by the enterohepatic circulation.

In this clinical study we present the long-term results of all patients who received systemic immunosuppression with MMF after keratoplasty from April-2005 to December-2007 and evaluate the efficacy and safety of this immunomodulatory drug.

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METHODS

Patient Selection

A total of 99 patients who had undergone penetrating keratoplasty were enrolled in this prospective study, 40 patients did not receive immunosuppressive treatment (control group), and 59 patients were treated postoperatively with MMF at a fixed dosage of 2 g (1 g twice daily). Exclusion criteria for the immunosuppressive treatment were: hypersensibility to MMF or MPA, pregnancy and breastfeeding. In all the cases, the final choice of immunosuppressive treatment was made by the patient after explaining the probability of graft rejection. Control group included mainly low-risk keratoplasties, patients who did not accomplish inclusion criteria, and eight high-risk keratoplasties that refused immunosuppressive treatment. The MMF treated group included all the high-risk ones except the eight patients mentioned above and patients with low-risk keratoplasties that wanted to be treated. The risk of graft rejection was defined into four categories: group 1 including avascular central corneal thinning, scarring or edema surrounded by healthy corneal tissue with excellent prognosis (90% or more); group 2 with lesions that extend partially or totally to periphery with an adequate surface and mild-to-moderate vascularity with very good prognosis (80-90%); group 3 with active diseases, extremes of corneal thickness and perforations with fair prognosis (50-80%); and group 4 with severe fibrovascular replacement of cornea, or previous history of graft rejection, with poor prognosis (0-50%).

Complete medical history was taken, and physical and laboratory examinations were performed for each patient.

Graft and Surgery

Once a donor was selected as suitable by the hospital transplantation group, grafts were obtained and preserved in Optisol medium. Time of storage was 52±17 hours in the MMF group and 68±21 hours in the control group. The human ABO type of donor and recipient was known and matched in all the patients. Penetrating keratoplasty was performed exclusively by three experienced surgeons according to standardized procedures: A Hessburg-Barron trephine was used for trephination. The graft was cut from the endothelial side, the recipient cornea centrally from the epithelial side 0.25 mm larger than patient trephine (grafts’ size was 7,75 mm and 8,25 for retransplantation cases) The graft was fixed using eight nylon 10-0 sutures and a running diagonal suture (Figure 1).

The eight nylon sutures were removed after topical anesthesia with proxymetacaine eye drops between the
second and third month after keratoplasty, the running diagonal suture was not removed before the twelfth month.

Treatment

Since 1984 we have administered topical corticosteroids postoperatively four times per day, tapered over 6 months. In high-risk keratoplasties, systemic corticosteroids (prednisone) and cyclosporine A (CsA) were combined.

Since 2001 we have avoided the use of CsA because of its systemic side effects, and have used instead systemic MMF at a fixed dosage of 2 g (1g twice daily).

Follow-Up

All patients were monitored for evidence of immune reactions, and clear graft survival during the study period (32 months). The occurrence of systemic side effects was also observed. Postoperatively examination was performed after the first week, and one, three, six months and then yearly, including visual acuity, slit-lamp examination of clear graft and anterior chamber, existence of epithelial alterations and suture.

Blood monitoring and MMF blood levels (therapeutic range 3-5 micrograms) were performed in all the patients treated with MMF in 3, 6 and 12 months after surgery.

Statistical Analysis

Metrical data of treated and non-treated groups were compared using analysis of chi-square test (patient age and gender, postmortem time and organ culture period). Evaluation for statistical significance was made using the log-rank test. A p value less than 0.05 was considered to show a statistically significant difference.

The probability of graft rejection was studied using a logistic regression model with two clinical variables:

risk level of graft rejection and MMF treatment. For this purpose we have employed the glmfit function, from MatLab 7.0.

RESULTS

We found no statistically significant differences between the two groups concerning patient age, gender and postmortem time of the grafts (Table 1).

Regarding previous corneal pathology, the main cause for keratoplasty was corneal edema after cataract surgery, followed by herpes, corneal ectasy and previous rejection graft (see table 1).

Graft rejection and time of its appearance was similar in the control group (13,5±9,61 months) and the treated one (11,4±5,74 months). According the risk classification, graft rejection appeared at 13±7,95 months in the risk group 1-2 and at 11,3±6,94 months in the group 3-4. We found that graft rejection percentage was greater in the group of non-treated patients (22.5%) than in the MMF-treated one (11.86%) (Table 2).

Data obtained from the estimation of the logistic regression model can be observed in table 3 and Chart 1. We find a highly significant difference in the probability of graft rejection from risk level 1 to 4. So, the

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<th>Table 1: Recipient eyes data</th>
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<td>Keratoplasties</td>
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<td>Male/female</td>
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<td>Age(&lt;65/&gt;65yr)</td>
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<td>Mean range culture time</td>
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<td>Corneal dystrophy</td>
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<td>Previous graft failure</td>
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MMF: mycophenolate mofetil.

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<th>Table 2: Graft rejection relationship with risk level and MMF treatment</th>
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odds of a graft rejection in a patient with risk level of 4 is 12.61 times greater than for a patient with risk level of 1, despite of having used or not immunosuppressive agents (\(e^{2.5348} = 12.61\)). The use of immunosuppressive agents has also an important effect on graft rejection probability for any risk level. It points out that using MMF reduces the odds of graft rejection in 77.72% (\(e^{-1.5015} = 0.2228 = 1 - 0.7772\)).

Blood monitoring of the patients treated with MMF, showed no significant changes in leucocytes number, and no increase in urea and creatinine levels.

The following side effects were noticed in the MMF group: gastrointestinal disturbances that appeared early in 10 cases within the first month and disappeared spontaneously, 3 cases of dermatitis within the third month, and 8 cases of astenia and sleepiness after six months of treatment. All these side effects were light and well tolerated. Only in six cases, premature withdrawal of the drug was judged necessary: three patients developed corneal graft infection (Figure 2), 2 patients severe respiratory infections and one patient developed a Hodgkin lymphoma.

**DISCUSSION**

The term «high-risk» is frequently applied to grafts known to have an increased likelihood of graft-rejection, but there is not a universally accepted definition of a high-risk cornea. The usual risk factors that predispose to graft rejection, include recipient vascularisation (two or more quadrants), previous graft failure, and the aetiology of the original corneal disease. Prognosis of normal risk keratoplasty is excellent, clear graft survival is up to 90% in the first two years even without systemic immunosuppression. These results can be attributed to the immune privilege of the cornea and the anterior chamber, the so called anterior chamber associated immune deviation (ACAID), firstly studied in the late 1800s by Van Dooremaal, and then by Medawar in the mid 1900s to fit in with emerging concepts of transplantation immunology. However, a dramatically poorer prognosis can be observed in high-risk situations, up to 75% of grafts experience rejections. In our study, after using a logistic regression model, we have noticed that from risk level 1 to risk level 4, the odds of graft rejection is 12.61 times greater (adjusted odds ratio 12.61, \(P=0.053\)).

Corneal graft failure subsequent to graft rejection remains an important cause of blindness and hence the need for developing new strategies for suppressing graft rejection is colossal. Since the introduction of systemic immunosuppression with Cyclosporine A in the postoperative treatment of high-risk keratoplasties, graft prognosis in such situations has improved considerably, but this therapeutic regimen comes with a high range of side effects and cost intensive follow up. The requirement for an effective, minimally toxic immunosuppressive agent is a major obstacle to performing high risk transplantation. MMF has been shown to be an effective immunosuppressive agent with a wide therapeutic range following renal transplantation. This study has evaluated the efficacy of MMF in preventing corneal allograft rejection and its safety with few side effects, mainly gastrointestinal disturbances. In case of development of ocular or respiratory infection, we advice immediate withdrawal of the drug, these infections can be caused or extended by the immunosuppression properties of the drug like any other immunosuppressive agent. Given the short period between keratoplasty and detection of one Hodgkin lymphoma case (one month), it may have been coincidental. MMF is not an alkylating agent and does not cause DNA miscoding, the manifestation of this disease could also be attributed to a general systemic immunosuppression.

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 (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 post-operative immunosuppression are reduced as a result of the omission of monitoring drug tires.

CONCLUSION

Although the cornea is classically described as possessing immunological privilege, the protection this affords is only relative and rejection is still the commonest cause of corneal graft failure. Consequently immunosuppression is still routinely used in keratoplasty. In the majority of grafts topical corticosteroids provide enough immunosuppression, but in high-risk grafts other therapeutic agents may be required. In this study, we present the results of our casuistry after using MMF as immunosuppressive agent. Using MMF reduces the odds of graft rejection in 77.72%, mainly after high-risk keratoplasty. MMF is safe with few side effects, and effective in preventing graft rejection. The wide therapeutic range and the omission of drug monitoring make this compound especially interesting for ophthalmic patients, representing a promising alternative therapeutic option in patients who are at a high risk for corneal graft failure; and should therefore be further investigated in controlled long term studies.

REFERENCES