

Topical administration of tacrolimus and corticosteroids with concentration gradients is effective in preventing immune rejection in high-risk keratoplasty: a 5-year follow-up study

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Research article

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Abstract

BACKGROUND: To evaluate the efficacy of the topical administration of immunosuppressants and corticosteroids with concentration gradients in the management of patients with high-risk keratoplasty.

METHODS: One hundred and six patients treated with topical immunosuppressants (50 eyes in the FK506 group and 56 eyes in the CsA group) and corticosteroid eye drops with concentration gradients were enrolled in the study. The rates of rejection episodes, irreversible rejection, graft survival, and related influential factors were evaluated.

RESULTS: The mean follow-up period was 48.1 ± 7.9 months (range, 36-60 months). The rates of rejection episodes ($P=0.043$) and irreversible rejection ($P=0.062$) were 14.0% and 6.00% in the FK506 group and 37.5% and 7.1% in the CsA group, respectively.

Kaplan-Meier survival analysis demonstrated a significantly higher graft survival rate in the FK506 group ($81.6\% \pm 5.3\%$, $71.1\% \pm 6.3\%$) compared with that in the CsA group ($71.1\% \pm 6.3\%$, $57.5\% \pm 7.5\%$) at 3 and 5 years after surgery ($P=0.006$). Multivariate logistic regression revealed that poor medication compliance with a preoperative risk score ≥ 3 ($P=0.016$) and endothelial immune rejection ($P=0.033$) were risk factors associated with graft survival.

CONCLUSIONS: Topical administration of tacrolimus and corticosteroids with concentration gradients is effective in decreasing the incidence of immune rejection in high-risk keratoplasty. Careful instruction of patients on the reasonable use of topical tacrolimus is critical to avoid immune rejection induced by sudden discontinuation of medication.

Background

Immune rejection after corneal transplantation remains the leading cause of graft failure. The 5-year survival rates of corneal grafts decreases dramatically in high-risk keratoplasty, ranging between 25% and 65%.¹⁻⁵ Although corticosteroids are currently the mainstay of treatment for routine postoperative management, they are insufficient in preventing graft rejection in high-risk patients.⁶ For such cases, a variety of systemic immunosuppressants have been administered to prevent or reverse immune rejection. Nonetheless, these medications can be associated with severe side effects such as nephrotoxicity, hepatotoxicity, hypertension, and altered glucose metabolism.⁷⁻⁹ Thus, topical administration of immunosuppressants like tacrolimus (also named FK506) and cyclosporine (CsA) is preferred. But limited data exist on the efficacy of topical immunosuppressants in preventing immune rejection in high-risk keratoplasty.¹⁰⁻¹³ In this study, we aimed to evaluate the efficacy of topical tacrolimus and CsA with 5 years of follow-up, and concluded topical administration of tacrolimus and corticosteroids eye drops with concentration gradients is effective in preventing immune rejection in high-risk keratoplasty.

Methods

PATIENTS: Topical 0.1% tacrolimus eye drops (Senju Pharmaceutical Ltd) is not currently approved for use in the treatment of immune rejection. Accordingly, the potential benefits and complications of off-label topical tacrolimus treatment were fully described to, and written informed consent was obtained from, all patients before participation in the study. This study was approved by the Institutional Review Board of Shandong Eye Hospital and adhered to the tenets of the Declaration of Helsinki.

106 eyes (106 patients) undergoing high-risk keratoplasty at the Shandong Eye Hospital were recruited on a consecutive basis from Jan 2013 to Jan 2015. Preoperative risk score for all the patients were recorded according to the method reported by Sloper CM et al.¹⁴ The patients were divided into two groups in accordance with their own wish, one is the FK506 group treated with 0.1% tacrolimus and corticosteroids eye drops, and the other is CsA group treated with 1% CsA (North China Pharmaceutical Group Corporation, NCPC) and corticosteroids eye drops. Patients with untreated glaucoma, cataract, or retinal detachment were not included.

Diagnosis of immune rejection on the basis of the following signs and symptoms: (1) ocular pain, photophobia, redness, and tearing; (2) rapid decrease in visual acuity; (3) combined graft edema and opacity, aggravated congestion in the recipient bed, and new blood vessels rapidly entering the graft periphery, or white infiltration at the sutures (negative results in corneal scraping and fungal and bacterial cultures), or effusion at the recipient-host interface in eyes treated by lamellar keratoplasty (LK); (4) endothelial rejection line or keratic precipitates; and (5) increased inflammatory cells in the anterior chamber.¹⁵

POSTOPERATIVE THERAPY: The treatment strategy of systemic and topical corticosteroid therapy for all patients were consistent. All patients received intravenous methylprednisolone (2 mg/kg) daily for 5 days. Oral prednisolone (1 mg/kg) was then started daily and tapered over a period of 2 to 3 months. Topical 1% Prednisolone Acetate eye drops was used 4 times per day for 1 month. Afterwards, 0.1% fluorometholone was used 4 times daily for 6 months and tapered to 0.02% fluorometholone three times daily for at least 1 year. Tobramycin and dexamethasone ophthalmic ointment was administered every night for 6 months and tapered to twice weekly.¹⁵⁻¹⁷ In addition, 0.1% tacrolimus eye drops or 1% CsA eye drops was given 4 times per day for 1 month and tapered to three times for 6 months and twice for at least 1 year.

ANTIREJECTION THERAPY: When immune rejection occurred, intravenous methylprednisolone (2 mg/kg) was given daily for 5 to 7 days. Oral prednisolone (1

mg/kg) was then started daily and tapered over a period of 1 to 2 months. Tobramycin and dexamethasone eyedrops were administered every 2 hours for the first 3 days and tapered to 4 times per day for the next 2 to 3 weeks. Afterward, 0.02% fluorometholone eyedrops were applied 4 times daily. Tobramycin and dexamethasone ophthalmic ointment was used every night for 1 month and tapered to twice weekly.¹⁵⁻¹⁷ Meanwhile, 0.1% tacrolimus eye drops or 1% CsA eye drops were given 4 times per day.

MAIN OUTCOME MEASURES: Patient history, demographic information, preoperative risk factors, onset time of immune rejection, symptoms, and medication compliance were collected. Complete ocular examinations were performed, including best-corrected visual acuity (BCVA), intraocular pressure and slit-lamp examination.

STATISTICAL ANALYSES: All data were described as mean values \pm standard deviation. Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, Illinois, USA). A *P* value of ≤ 0.05 was considered statistically significant. The demographics and preoperative risk score were compared with the Wilcoxon signed ranks test between the two groups. The rejection episodes and irreversible rejection (loss of graft transparency) in each group were analyzed using the Mann-Whitney U test. Kaplan-Meier survival analysis and log rank tests were performed to evaluate the graft survival. The influential factors, including age, gender, preoperative risk score (≥ 3 or < 3), surgical treatment (penetrating keratoplasty or keratolimbal allograft), topical immunosuppressants (0.1% tacrolimus eye drops or 1% CsA eye drops), poor medication compliance with 0.1% tacrolimus eye drops or 1% CsA eye drops, type of immune rejection (endothelial and non-endothelial immune rejection) were analyzed using multivariate adjusted logistic regression.

Results

The mean follow-up period was 48.1 ± 7.9 months (range, 36–60 months). Sixty eight patients were male, and forty eight patients was female. The mean age was 49.7 ± 12.2 years (range, 11–70 years). The demographics and preoperative risk score of the two groups were comparable and intergroup comparisons showed no statistical differences (Table 1).

REJECTION EPISODES: In FK506 group, immune rejection was observed in 7 eyes, with stromal rejection in 3 eyes and endothelial rejection in 4 eyes, the rate of rejection episodes was 14.0% (7/50). The causative factors was stopping taking FK506 eye drops in 7 eyes (with the average interval of 4.9 ± 0.2 days). Rejection occurred within 6 months after surgery in 4 eyes, at 6 months to 1 year in 1 eyes, at 1 year to 2 years in 1 eye, and at 2 years to 3 years in 1 eyes. 4 eyes restored a clear graft after 7.9 ± 1.40 days of antirejection therapy. However, the corneal grafts showed continuously edematous and opaque in 3 eyes. The rate of irreversible rejection was 6.00%.

In CsA group, immune rejection was observed in 21 eyes, with stromal rejection in 4 eyes and endothelial rejection in 17 eyes. The rate of rejection episodes was 37.5% (21/56), and the difference was statistically significant (*P* = 0.043) between the two groups. The causative factors included poor compliance with medications in 5 eyes, stopping taking corticosteroids eye drops in 6 eyes (with the average interval of 8.4 ± 2.3 days), and stopping taking CsA eye drops in 10 eyes (with the average interval of 6.3 ± 2.5 days). Rejection occurred within 6 months after surgery in 8 eyes, at 6 months to 1 year in 7 eyes, at 1 year to 2 years in 3 eye, and at 2 years to 3 years in 3 eyes. 17 eyes restored a clear graft after 8.8 ± 2.2 days of antirejection therapy. However, the corneal grafts showed continuously edematous and opaque in 4 eyes.

The rate of irreversible rejection was 7.1%, and there was no statistically significant difference ($P = 0.062$) between the two groups.

GRAFT SURVIVAL: The graft survival was $81.0\% \pm 7.4\%$, $72.0\% \pm 8.9\%$ at 3 years after surgery, and $71.9\% \pm 6.2\%$, $61.2\% \pm 6.9\%$ at 5 years after surgery in FK506 group and CsA group, respectively (Fig. 1). Kaplan-Meier analysis and log rank tests showed that patients in FK506 group had a significantly higher graft survival rate both at 3, 5 years after surgery than patients in CsA group, and the difference was statistically significant ($P = 0.000$).

Table 1
Preoperative characteristics of patients in FK506 group and CsA group

Characteristics	FK506 group	CsA group	<i>P</i>
No. Eyes	50	56	
Age, y			
Mean(SD)	51.2(11.9)	48.1(12.3)	0.521
Range	11–67	12–70	
Sex			
Male: Female	26:24	32:24	0.342
Preoperative Risk Factors			
Previous graft rejection	9	10	0.617
Stromal vascularization \geq two quadrants	9	9	1.000
Chemical burn	7	7	1.000
Grafts diameter \geq 9 mm	14	16	0.562
Infectious keratitis and corneal perforation	11	14	0.430
Surgical Treatment			
Penetrating keratoplasty	45	51	0.571
Keratolimbal allograft	5	5	1.000

INFLUENTIAL FACTORS: The graft survival was correlated with preoperative risk score (≥ 3 , $P = 0.016$), and endothelial immune rejection ($P = 0.033$) (Table 2).

Table 2
Influential factors for graft survival

Variable	No. Eyes	P value	RR(95% CI)
Age		0.746	1.208(0.385,3.786)
Sex	68	0.739	0.708(0.093,5.400)
Male	48		
Female			
Preoperative Risk Score	28	0.016	4.161(1.307,13.250)
≥ 3	78		
< 3			
Surgical Treatment	96	0.812	0.770(0.090,6.621)
Penetrating keratoplasty	10		
Keratolimbic allograft			
Topical Immunosuppressants	56	0.676	0.964(0.109,7.121)
1% CsA	50		
0.1% Tacrolimus			
Poor Medication Compliance	10	0.604	1.667(0.131,7.551)
1% CsA	7		
0.1% Tacrolimus			
Type of Immune Rejection	21	0.033	3.532(1.109,11.251)
Endothelial	7		
Non-endothelial			

SIDE EFFECTS: The common side effect was redness (15 eyes), burning (14 eyes), and stinging (14 eyes) sensation on drug instillation, which were more often occurred in CsA group (redness in 8 eyes, burning in 9 eyes, and stinging in 9 eyes). No cataracts or elevation of intraocular pressure were detected in the two groups during the follow-up.

Discussion

High-risk keratoplasty have been defined as having at least two quadrants of stromal vascularization and/or a history of previous graft rejection. Other risk factors include chemical burn, corneal grafts diameter exceeding 9 mm, perforation or ocular inflammation at the time of surgery, and low recipient age.^{13,14,18,19} Immune rejection in high-risk keratoplasty remain a therapeutic challenge to eye doctors.

They made lots of efforts on variety and usage of anti-rejection medications,^{4,11,12,20} but still lacked an ideal treatment strategy. In our series, we applied 0.1% tacrolimus and corticosteroids eye drops with concentration gradients to patients with high-risk keratoplasty, and concluded it is effective in reducing immune rejection and prolonging graft survival.

Corticosteroid therapy remain the mainstay of preventing corneal graft rejection, for its dramatic inhibition of dendritic cell (DC) differentiation and maturation, and restoring a noninflamed microenvironment to support the transplanted graft.⁵ But the regimen varies widely among respondents in the Cornea Society survey. Long-term use of corticosteroid eye drops is not recommend due to its underlying side events such as increased intraocular pressure or cataracts. Given that the peak time of immune rejection was 1 to 3 months after high-risk keratoplasty,¹⁵ intensive topical and intravenous steroids were given within 1 month after surgery, and tapered over a period of 2 months. Afterwards, fluorometholone was prescribe and tapered to a maintenance dose. Fluorometholone eye drops rapidly form inactive metabolites in the corneal tissue, and only a small portion passes through the cornea into the aqueous humor,²¹ thus reducing the possibility of side effects associated with elevations in intraocular pressure or cataracts. Unlike the results of Zhai et al, rejection was observed after 30 months after surgery in 4 eyes in our study, making irreparable loss of graft endothelial cell.²² Therefore, we advocate maintenance dose of corticosteroid eye drops during the follow-up period, and regular detection of intraocular pressure is worthwhile.

Tacrolimus and CsA are calcineurin-blocking drugs that inhibit clonal expansion of T lymphocytes through binding of intracellular proteins called immunophilins.²⁰ Topical CsA has been prescribed for years to treat different immune diseases of the eye. However, majority of prospective studies have failed to demonstrate any benefit with the use of topical CsA for high-risk keratoplasty.^{4,12,23} Tacrolimus has been extensively used in preventing immune rejection for human organ transplantation. But few case series have reported beneficial effect of topical tacrolimus in human high-risk corneal transplantation. In this study, the rate of rejection episodes in FK506 group was much lower than that in CsA group ($P=0.043$). Moreover, the graft survival was significantly higher both at 3, 5 years after surgery when comparing with the study conducted by Chow et al.¹⁹ Systemic side effects on blood pressure, renal, and liver function were avoided consequently. A small sample size is one limitations of our study, which may influence the treatment response. Further investigation on a larger sample seems to be necessary to evaluate the medication efficacy.

The efficacy of tacrolimus as an immunosuppressive agent is 10–100 times higher than that of CsA,²⁰ but sudden discontinuation of tacrolimus is more likely to induce immune rejection, with an average interval of 4.9 ± 0.2 days (shorter than that of CsA), which is a problem that can not be ignored. In this study, the causative factors for immune rejection included poor compliance or stopping taking medications without doctors' recommendations, thus careful medicine instructions to patients can be helpful. The restoration of corneal clarity after immune rejection is directly related to the interval from symptom onset to treatment and the degree of immune response. The longer the interval is and/or the

severer the endothelial rejection is, the harder it will be to restore corneal transparency ($P= 0.033$). In addition, there was no statistically significant difference in the rate of irreversible rejection ($P= 0.062$) between the two groups. This is consistent with the reported study by Hashemian et al, in which topical tacrolimus can decrease the recurrence of rejection. However, it may not improve rejection reversal success.

Conclusion

In conclusion, topical administration of tacrolimus and corticosteroids with concentration gradients is effective in decreasing the incidence of immune rejection, and significantly prolonging graft survival in high-risk keratoplasty. It is critical to recommend reasonable use of topical tacrolimus to patients, thus avoiding inducing immune rejection by stop taking in sudden.

Abbreviations

Tacrolimus: FK506

Cyclosporine: CsA

lamellar keratoplasty: LK

Best-corrected visual acuity: BCVA

Dendritic cell: DC

Declarations

Ethics approval and consent to participate: Ethics approval was obtained from the Ethics Committee of Shandong Eye Hospital. Written informed consents were obtained where participants are children (under 16 years old) from their parents.

Consent for publication: All authors have reviewed the final version of the manuscript and approve it for publication.

Availability of data and material: All data and material in the manuscript are available.

Competing interests: No competing interests were exist in the study.

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Authors' contributions: Design and conduct of the study (XQ, HG); Collection of the data (LW, XZ, ML); Management, analysis, and interpretation of the data (LW, HG); Preparation of the manuscript (XQ); Review and approval of the manuscript (HG)

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Figures

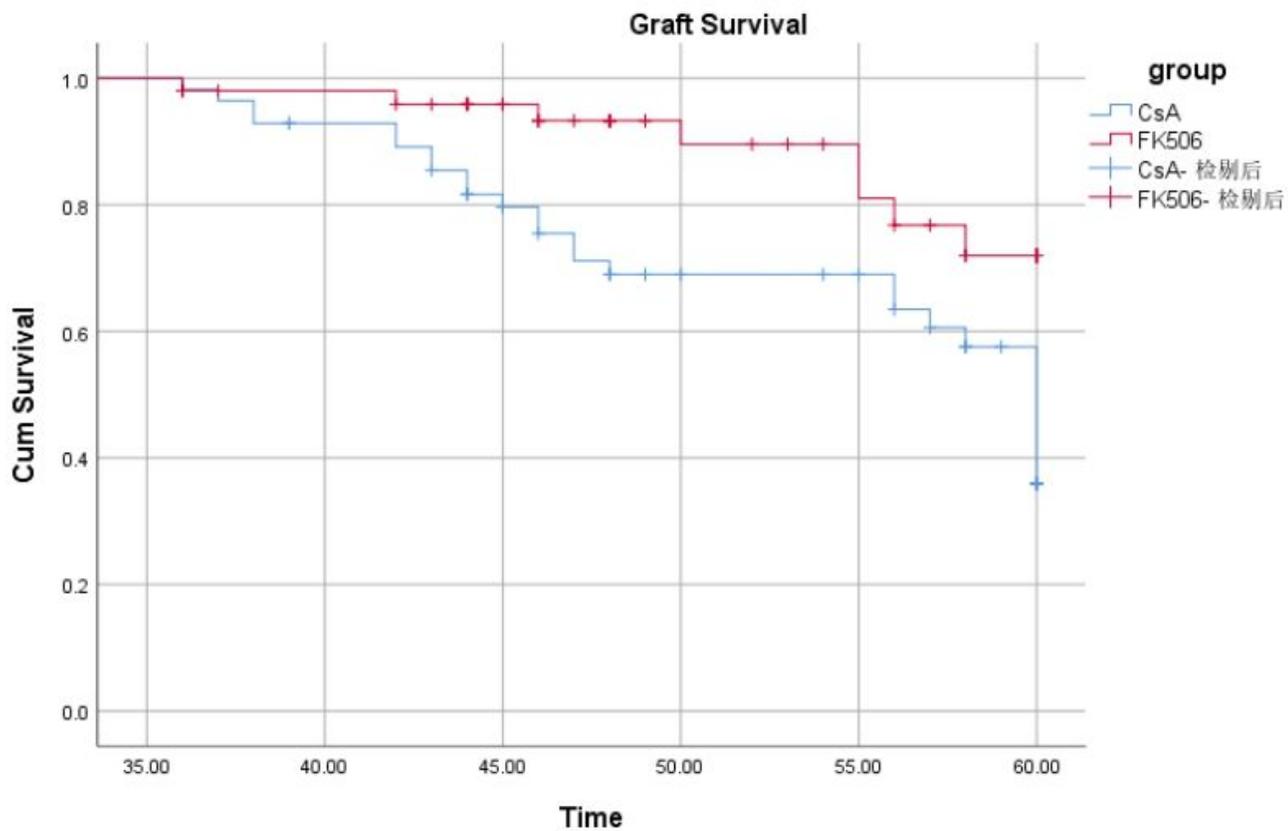


Figure 1

Kaplan-Meier curve of graft survival rate of FK506 group and CsA group at 3, 5 years after surgery.