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%±5.3%, 71.1%±6.3%) compared with that in the CsA group (71.1%±6.3%, 57.5%±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 are dramatically decreased for high-risk keratoplasty and range 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 such as tacrolimus (also named FK506) and cyclosporine (CsA) is preferred. However, limited data exist on the efficacy of topical immunosuppressants in preventing immune rejection after high-risk keratoplasty.[10-13] In this study, we aimed to evaluate the efficacy of topical tacrolimus and CsA during 5 years of follow-up and concluded that topical administration of tacrolimus and corticosteroid eye drops with concentration gradients is effective in preventing immune rejection in high-risk keratoplasty.

Methods

PATIENTS: Topical eye drops containing 0.1% tacrolimus (Senju Pharmaceutical Ltd) are 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, and written informed consent was obtained from all patients before participation in the study. This study was approved by the Institutional Review Board of Eye Hospital of Shandong First Medical University and adhered to the tenets of the Declaration of Helsinki.

A total of 106 eyes (106 patients) undergoing high-risk keratoplasty at Eye Hospital of Shandong First Medical University were recruited on a consecutive basis from Jan 2013 to Jan 2015. The preoperative risk score for all patients was recorded according to the method reported by Sloper CM et al.[14] The patients were divided into two groups in accordance with their desired treatment: the FK506 group was treated with 0.1% tacrolimus and corticosteroid eye drops, and the CsA group was treated with 1% CsA (North China Pharmaceutical Group Corporation, NCPC) and corticosteroid eye drops. Patients with untreated glaucoma, cataracts, or retinal detachment were not included.

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

POSTOPERATIVE THERAPY: The treatment strategy used for systemic and topical corticosteroid therapy for all patients was 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 were 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 were administered every night for 6 months and tapered twice weekly.[15-17] In addition, 0.1% tacrolimus eye drops or 1% CsA eye drops were administered 4 times per day for 1 month and then tapered to three times a day for 6 months and twice a day for at least 1 year. For patients with dry eye, 0.3% sodium hyaluronate eye drops or carbomer gel was given accordingly.

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 then 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 then tapered to twice weekly use.[15-17] Meanwhile, 0.1% tacrolimus eye drops or 1% CsA eye drops were used 4 times per day.

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

STATISTICAL ANALYSES: All data are described as the mean value ± 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 rank 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 graft survival. The most highly influential factors, including age, gender, preoperative risk score (≥ 3 or < 3), surgical treatment (penetrating keratoplasty or keratolimbal allograft), use of topical immunosuppressants (0.1% tacrolimus eye drops or 1% CsA eye drops), poor medication compliance when using 0.1% tacrolimus eye drops or 1% CsA eye drops, and type of immune rejection (endothelial or nonendothelial 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 were female. The mean age was 49.7±12.2 years (range, 11-70 years). The demographics and preoperative risk scores of the two groups were comparable, and the intergroup comparisons showed no significant differences (Table 1).

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

Characteristics	FK506 group	CsA group	*P*
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 ≥ two quadrants	9	9	1.000
Chemical burn	7	7	1.000
Grafts diameter ≥ 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

REJECTION EPISODES: In the FK506 group, immune rejection was observed in 7 eyes, with stromal rejection in 3 eyes and endothelial rejection in 4 eyes, and the rate of rejection episodes was 14.0% (7/50). The causative factor was discontinuation of the FK506 eye drops in 7 eyes (with an 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 eye, at 1 year to 2 years in 1 eye, and at 2 years to 3 years in 1 eye. The graft in four eyes was restored to a clear graft after 7.9±1.40 days of antirejection therapy. However, the corneal grafts were continuously edematous and opaque in 3 eyes. The rate of irreversible rejection was 6.00%.

In the 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, discontinuation of the use of corticosteroid eye drops in 6 eyes (with an average interval of 8.4±2.3 days), and discontinuation of the use of CsA eye drops in 10 eyes (with an 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 eyes, and at 2 years to 3 years in 3 eyes. The graft in

seventeen eyes was restored to a clear graft after 8.8±2.2 days of antirejection therapy. However, the corneal grafts were 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 rate was 81.0%±7.4% and 72.0%±8.9% at 3 years after surgery and 71.9%±6.2% and 61.2%±6.9% at 5 years after surgery in the FK506 group and CsA group, respectively (Figure 1). Kaplan-Meier analysis and log rank tests showed that patients in the FK506 group had a significantly higher graft survival rate at both 3 and 5 years after surgery than patients in the CsA group, and the difference was statistically significant (*P*=0.000).

INFLUENTIAL FACTORS: Graft survival was correlated with the 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		
Keratolimbal 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 effects were redness (15 eyes), burning (14 eyes), and a stinging sensation (14 eyes) after drug instillation, which occurred more often in the CsA group (redness in 8 eyes, burning in 9 eyes, and stinging in 9 eyes) than in the FK506 group. No cataracts or elevation of intraocular pressure were detected in the two groups during the follow-up.

Discussion

High-risk keratoplasty is defined as having at least two quadrants of stromal vascularization and/or a history of previous graft rejection. Other risk factors include chemical burns, corneal graft diameters

exceeding 9 mm, perforation or ocular inflammation at the time of surgery, and a low recipient age.[13,14,18,19] Immune rejection in high-risk keratoplasty remains a therapeutic challenge for eye doctors. Doctors have made many efforts to examine the variety and usage of anti-rejection medications,[4,11,12,20] but there is still a lack of an ideal treatment strategy. In our study, we applied 0.1% tacrolimus and corticosteroid eye drops with concentration gradients to patients with high-risk keratoplasty and concluded that they are effective in reducing immune rejection and prolonging graft survival.Corticosteroid therapy remains the mainstay method of preventing corneal graft rejection because of its dramatic inhibition of dendritic cell (DC) differentiation and maturation and restoration of a noninflamed microenvironment to support the transplanted graft.[5] However, the regimen used among respondents in the Cornea Society survey varied widely. Long-term use of corticosteroid eye drops is not recommended due to 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 administered within 1 month after surgery and tapered over a period of 2 months. Afterwards, fluorometholone was prescribed and tapered to a maintenance dose. Fluorometholone eye drops rapidly formed inactive metabolites in the corneal tissue, and only a small proportion passed through the cornea into the aqueous humor,[21] thus reducing the possibility of side effects associated with the elevation of intraocular pressure or cataracts. Unlike the results of Zhai et al., rejection was observed 30 months after surgery in 4 eyes in our study, resulting in irreparable loss of graft endothelial cells.[22] Therefore, we advocate the use of a 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 intracellular proteins called immunophilins.[20] Topical CsA has been prescribed for years to treat different immune diseases of the eye. However, the majority of prospective studies have failed to demonstrate any benefit from 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. However, few case series have reported the beneficial effects of topical tacrolimus in human high-risk corneal transplantation. In this study, the rate of rejection episodes in the FK506 group was much lower than that in the CsA group (P=0.043). Moreover, graft survival was significantly higher at both 3 and 5 years after surgery compared with that in the study conducted by Chow et al.[19] Systemic side effects on blood pressure, renal function, and liver function were consequently avoided.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 was not observed in the study of Zhai et al.[22] In addition, the restoration of corneal clarity after immune rejection is directly related to the interval from symptom onset to treatment and the degree of the immune response. The longer the interval is and/or the more severe the endothelial rejection is, the harder it will be to restore corneal transparency (P=0.033). There was no statistically significant difference in the rate of irreversible rejection (P=0.062) between the two groups. This is consistent with the study reported 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 the reasonable use of topical tacrolimus to patients, thus avoiding inducing immune rejection by sudden discontinuation of medication. Further randomized controlled trials are required to provide more objective evidence to evaluate medication efficacy.

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 to publish: 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 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

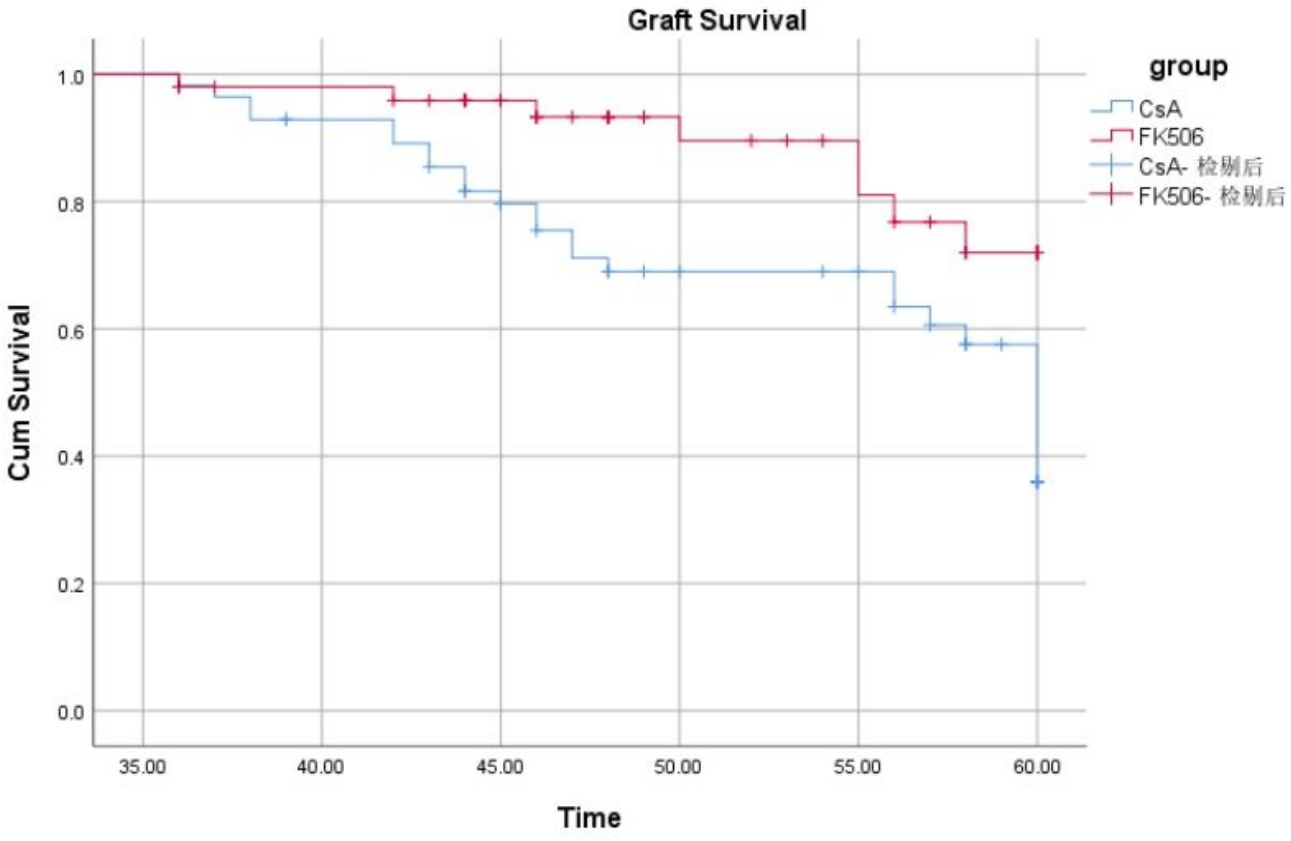

Figure 1

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