International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.701.707

Research Article
Comparative Study of Staged Optimized Immunosuppressive Regimen With Conventional Regimen in High-Risk Corneal Transplantation

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Abstract

Background and Objective: Corneal transplantation, also known as keratoplasty, is a common surgical procedure for treating various corneal diseases and injuries. This study aimed to compare the efficacy and safety of a staged optimized immunosuppressive regimen with a conventional regimen in high-risk corneal transplant patients. **Materials and Methods:** Two hundred patients were randomly assigned to either the treatment group (n=100) or the control group (n=100). The treatment group received a staged optimized immunosuppressive regimen, while the control group received a conventional regimen. The study measured and compared the rejection, safety profile and improvement of visual acuity. **Results:** The treatment group exhibited a lower rejection rate ($p<0.05$), a longer time to the first rejection episode ($p<0.05$) and a higher graft survival rate ($p<0.05$) compared to the control group. The optimized anti-rejection treatment regimen did not increase the incidence of infections, elevated intraocular pressure, or cataract formation ($p<0.05$). The percentage of improved visual acuity in the research and control groups was 85 and 75%, respectively. **Conclusion:** It was found that a staged optimized immunosuppressive regimen for high-risk corneal transplant patients was associated with superior efficacy in terms of reduced rejection rate, increased time to first rejection episode and improved graft survival rate compared to the conventional regimen.

Key words: Corneal transplantation, high-risk patients, anti-rejection therapy, graft survival, rejection rate

Citation: Zhang, C., Q. Kang and Y. Yang, 2023. Comparative study of staged optimized immunosuppressive regimen with conventional regimen in high-risk corneal transplantation. Int. J. Pharmacol., 19: 701-707.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cornea is the transparent anterior structure of the eye, which plays a crucial role in protecting the internal structures of the eye and in refraction and is a key component in maintaining normal vision[1]. Corneal diseases refer to a class of conditions that cause structural and functional damage to the cornea due to various reasons, such as infection, trauma, metabolic disorders, genetic factors or immune-related diseases[2,3]. Numerous corneal diseases have the potential to induce a pronounced diminishment in corneal transparency, provoking an aberrant refraction of light rays upon the delicate surface of the retina. Hence, the consequences may manifest as impaired vision or, in grave circumstances, an irreversible state of blindness[4]. Corneal transplantation is an effective method for treating severe corneal diseases, by replacing the damaged cornea with a healthy donor cornea, which restores the normal structure and function of the cornea and improves the patient's vision[5-7]. However, the occurrence of complications after corneal transplantation can affect the survival of the transplanted cornea and the most significant complication is rejection. The transplanted cornea may be recognized as a foreign body by the host immune system, leading to rejection reactions, which can damage or even cause loss of function of the transplanted cornea[8]. Therefore, the rational use of anti-rejection drugs is of great significance in preventing rejection reactions after corneal transplantation. This study aims to optimize the treatment regimen of anti-rejection drugs, reduce the incidence of complications after corneal transplantation and prolong the survival of the transplanted cornea.

MATERIALS AND METHODS

Study subjects: A total of 200 patients who underwent corneal transplantation in Chengdu Aidi Eye Hospital (Chengdu, China) from 2018 to 2021 were selected as the study population. The study enrolled participants who met specific inclusion criteria, which encompassed the following conditions: Being within the age range of 18 to 75 years old, having a severe corneal disease, undergoing their initial corneal transplantation and not exhibiting any other ocular diseases. The study excluded participants who had severe systemic diseases, immune system disorders, allergies or clear contraindications to anti-rejection medications. Additionally, patients who had previously used immunosuppressive agents or had undergone other ocular surgeries prior to the study were also excluded. According to the time of patient enrollment, 200 patients were randomly divided into the study group and the control group at a ratio of 1:1, with 100 patients in each group. During the treatment period, one patient in each group was dropped out due to severe allergic reactions to the medication. The baseline characteristics of the two groups of patients, including age, sex, transplantation indications and transplantation types, were statistically tested and no significant distinctions were noted between the two groups, as indicated in Table 1.

The Ethics Committee of Chengdu Aidi Eye Hospital granted approval for this study. All participants were informed about the objectives of the study and they signed the informed consent form.

Research methods

Control group and treatment plan: Following the surgical procedure: (1) A noteworthy phase involved the administration of cyclosporine eye drops as a standard systemic immunosuppressant. These drops were initially prescribed to be applied four times daily, maintaining this frequency throughout the first month after surgery. Subsequent to the patient's discharge from the hospital, a gradual decrement in the administration frequency of cyclosporine eye drops was implemented. The dosage frequency was reduced by one drop per month until reaching a minimum of once per day. This reduced frequency was subsequently maintained for a minimum duration of six months. Regular monitoring of blood drug concentration and liver and kidney function was performed during this period. (2) One-week post-surgery, the use of topical immunosuppressant, prednisolone eye drops, commenced. This was applied four times daily, with each dose consisting of one drop and continued for at least one year. (3) Upon the occurrence of graft rejection, the frequency of prednisolone eye drops was increased to six times a day, with each dose consisting of one drop. This regimen continued until the graft rejection symptoms subsided. (4) If the rejection reaction was severe or could not be controlled by increasing the frequency of prednisolone eye drops, the frequency of cyclosporine eye drops could be increased from the original four times a day to six times a day. Close monitoring of the blood drug concentration and liver and kidney function was required during this treatment phase to prevent adverse reactions. Once the rejection reaction was under control, the frequency of cyclosporine eye drops gradually returned to its original level.

Treatment group and treatment plan: (1) Immediately after surgery, patients started on a regimen of oral prednisolone (0.5 mg kg^{-1} day) and cyclosporine capsules (2 mg kg^{-1} day). Both medications were given orally, twice daily for the first month. (2) Concurrently, tacrolimus eye drops and

prednisolone eye drops were initiated three days post-surgery, applied four times daily and maintained for at least one year. After discharge, both types of eye drops were gradually tapered on a monthly schedule, with a reduction of one application per day each month until reaching once-daily application. (3) One month after surgery, individualized treatment plan adjustments were made according to the patient's rejection risk assessment results and immune monitoring indicators, such as CD4+/CD8+T cell ratio, interleukin (IL) levels, etc., adjust the immunosuppressive drug usage, dosage, frequency and combination therapy plan. The risk factors for rejection reaction in patients after corneal transplantation were shown in Table 2 and patients with two or more high-risk indicators are considered to have a higher drug rejection risk. (4) For high-risk patients, after the first month, the prednisolone and cyclosporine doses were increased to 1 mg kg^{-1} day and 3 mg kg^{-1} day, respectively. The frequency of tacrolimus and prednisolone eye drops was also increased to six times a day. In the case of a graft rejection episode, the frequency of eye drops could be further increased to eight times daily and the oral dose of prednisolone and cyclosporine temporarily raised to 1.5 mg kg^{-1} day and 3.5 mg kg^{-1} day, respectively until the episode was under control. (5) For low-risk patients, the oral prednisolone and cyclosporine doses were reduced gradually to 0.25 mg kg^{-1} day and 1.5 mg kg^{-1} day, respectively based on the patient's condition and immune status. The frequency of tacrolimus and prednisolone eye drops was also tapered to twice a day. (6) If a severe rejection episode occurred that couldn't be controlled by increasing the frequency of eye drops, the oral prednisolone and cyclosporine dose was temporarily raised to 1.5 mg kg^{-1} day and 3.5 mg kg^{-1} day, respectively until the episode was controlled. The medication was then gradually reduced back to its prior levels over the next month, while closely monitoring the patient's blood drug concentration and liver and kidney function.

Evaluation indicators rejection

Rejection rate: Within the span of one year after corneal transplantation, the occurrence of rejection reactions was examined in both the experimental and control groups. The lower the rejection rate, the more effective the treatment regimen. (2) Time to first rejection episode: The time elapsed from the date of corneal transplantation to the first occurrence of a rejection reaction. A longer time to the first rejection episode indicates a more effective treatment regimen in preventing early rejection reactions. (3) Graft survival rate: The proportion of successful corneal grafts in both research and control groups at the end of the 1-year follow-up period. A higher graft survival rate indicates a more effective treatment regimen in maintaining long-term graft function.

Safety profile: The incidence of adverse events related to the use of immunosuppressive agents, such as infections, renal and hepatic toxicity and other systemic side effects, in both research and control groups during the study period. A lower incidence of adverse events indicates a safer treatment regimen.

Improvement of visual acuity: Record the patient's uncorrected visual acuity or best-corrected visual acuity before corneal transplantation surgery. At the 12th month after corneal transplantation, measure the patient's visual acuity using the same type of visual acuity chart. Compare the patient's visual acuity data before and after surgery at various time points. If the patient's postoperative visual acuity improves by at least one visual acuity level (from 0.1 to 0.2), it is considered a significant improvement in visual acuity.

Case presentation: A 41-year-old man diagnosed with corneal leukoplakia and viral keratitis in his right eye underwent lamellar keratoplasty. Preoperatively, his right eye's corrected visual acuity was 0.06 with notable irregular white lesions on the cornea and significant neovascularization. One day post-operatively, the eye exhibited mild graft edema with a visual acuity of CF50CM. However, two years after surgery, the right eye showed a marked improvement with an uncorrected visual acuity of 0.4 and corrected visual acuity of 0.6, accompanied by a clear corneal graft and stable intraocular pressure (Fig. 1).

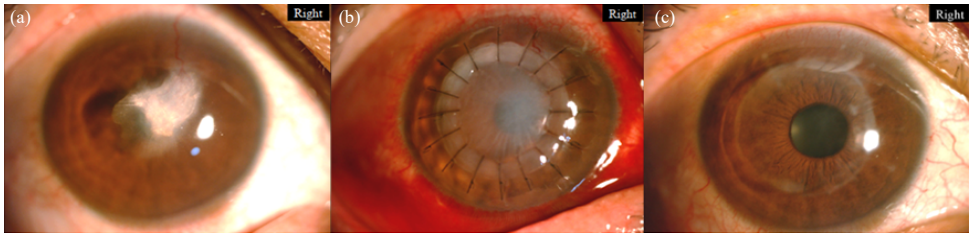

Fig. 1(a-c): Clinical observations of the presented case

Statistical analysis: In this study, it was attempted to employ the following statistical methods to analyze and compare the data collected from both research and control groups:

- **Descriptive statistics:** It was attempted to use the mean and standard deviation (SD) to describe continuous variables (such as age) and frequency and transplantation type)
- **Independent T-Test:** This test will be used to compare the means of continuous variables between the research and control groups (such as age). The results will be considered statistically significant if the p-value is less than 0.05
- **Chi-square Test:** This test will be used to compare the proportions of categorical variables between the research and control groups (such as gender and transplantation type). The statistical analysis was performed using SPSS 5.0 software and a p-value of less than 0.05 was considered statistically significant

RESULTS

Baseline characteristics of patients: The baseline characteristics between the treatment and control groups of patients were well-matched. Both groups had similar average ages (47.5 vs 48.2 years) and gender distributions (6237 male female in treatment vs 5940 in control). The reasons for requiring a graft, such as corneal inflammation, ulcer, malnutrition and scarring, were uniformly distributed across both groups. Additionally, the majority of patients in each group underwent penetrating keratoplasty, while a smaller portion underwent lamellar keratoplasty. Statistical analysis confirmed no significant differences between the two groups in these characteristics, indicating a balanced starting point for the study ($p>0.05$, Table 1).

Comparison of rejection rate between the two groups: The findings indicated that the treatment group, which followed an enhanced anti-rejection treatment regimen, exhibited a notably reduced rejection rate, an extended duration before the first rejection episode and a higher rate of graft survival versus the control group receiving conventional treatment ($p<0.05$, Table 3).

Exploring the differences in safety profile: These results suggested that the optimized anti-rejection treatment regimen in the treatment group did not lead to a significant increase in the incidence of infections, elevated IOP, or cataract formation compared to the conventional treatment in the control group ($p<0.05$, Table 4).

Table 1: Baseline characteristics of patients

Characteristics	Treatment group (n=99)	Control group (n=99)	t/Chi-square value	p-value
Age (years)	47.5±10.3	48.2±10.7	0.523	0.602
Gender (Male/Female)	62/38	59/41	0.210	0.647
Indications for graft			1.782	0.620
Corneal inflammation	45	43		
Corneal ulcer	30	33		
Corneal malnutrition	15	14		
Corneal scarring	10	10		
Graft type			0.141	0.707
Penetrating keratoplasty	80	82		
Lamellar keratoplasty	20	18		

$p<0.05$ is indicative of statistical significance and ± represents the standard deviation, reflecting the variability of the data around the mean

Table 2: Summarizing risk factors for rejection reaction in patients after corneal transplantation

Risk assessment indicator	High-risk criteria	Low-risk criteria
Age	≥60 years old	<60 years old
Duration of corneal lesion	≥5 years	<5 years
Number of transplant failures	≥2 times	<2 times or no transplant failure history
CD4+/CD8+ T cell ratio	>2.5	2.5
Interleukin (IL) level	Significantly elevated (e.g., >2 times the upper limit of normal)	Normal range or slightly elevated
HLA matching degree	<6/8 matching	<6/8 matching
Corneal transplant type	PKP or DALK	DSEK or DMEK
Other ocular surgery history	Presence of severe complications in other ocular surgery history	No significant complications in ocular surgery history

$p<0.05$ is indicative of statistical significance

Table 3: Comparison of rejection between the two groups

Outcome	Treatment group (n = 99)	Control group (n = 99)	t/Chi-square value	p-value
Rejection rate (%)	12	24	6.857	0.009
Time to first rejection episode (months)	8.6±3.1	6.2±2.8	5.862	<0.001
Graft survival rate at 1 year (%)	95	85	6.190	0.013

p<0.05 is indicative of statistical significance and ± represents the standard deviation, reflecting the variability of the data around the mean

Table 4: Comparison of safety profile between the research and control groups

Outcome (%)	Treatment group (n = 99)	Control group (n = 99)	t/Chi-square value	p-value
Infection rate	5	10	2.667	0.102
Incidence of elevated IOP	8	12	1.333	0.248
Incidence of cataract formation	15	20	1.176	0.278

p<0.05 is indicative of statistical significance

Comparison of improvement of visual acuity between the two groups: The treatment group had a higher percentage of patients with a significant improvement in visual acuity compared to the control group. The percentage of patients with improved visual acuity in the treatment group was 85%, while it was 75% in the control group. The chi-square value was 3.846, with a p-value of 0.05, indicating a statistically significant difference between the two groups.

DISCUSSION

The results of the present study demonstrated that the optimized immunosuppressive regimen significantly improved graft survival reduced the incidence of rejection episodes and resulted in better visual acuity outcomes compared to the standard immunosuppressive regimen. Furthermore, the safety profile was comparable between the two groups. In recent years, there has been a significant rise in the utilization of corneal transplantation as a prevalent method to address a range of corneal issues. These issues include corneal dystrophies, corneal scarring and corneal ulcers[9,10]. Despite advancements in surgical techniques and immunosuppressive therapies, high-risk patients still face significant challenges, including increased rates of graft rejection and lower graft survival. These high-risk patients often have a history of previous graft failure, severe ocular surface inflammation, or extensive corneal neovascularization, which may contribute to increased immune response and a higher likelihood of graft rejection[11-13]. Therefore, optimizing the immunosuppressive regimen for these patients is crucial to improving graft outcomes and overall patient satisfaction.

Prior research has examined a multitude of strategies to amplify the effectiveness of immunosuppressive protocols, incorporating different immunosuppressive medications and patient-specific treatment blueprints[14,15]. Nonetheless, a more focused and personalized approach for high-risk corneal transplant patients is yet to be found. The incorporation of orally administered prednisolone and cyclosporine, along with tacrolimus and prednisolone eye drops, into the treatment regimen proposed in this study signifies an encouraging development in bolstering the overall effectiveness of immunosuppressive strategies in various transplantation contexts[16]. The experimental group's treatment regimen in this study was designed to be more individualized and targeted, which significantly contributed to its increased efficacy over the control group's regimen. Firstly, the use of both systemic and topical medications (orally administered prednisolone, cyclosporine capsules and tacrolimus and prednisolone eye drops) in the experimental group not only targeted the systemic immune response but also directly influenced the local ocular immune environment, which enabled comprehensive immunosuppression. This combination was particularly crucial in high-risk patients where a robust and diverse immunosuppressive strategy is needed to prevent graft rejection. In contrast, the control group regimen primarily focused on topical therapy, which might not have been sufficient for such high-risk patients. Secondly, the treatment in the experimental group allowed for personalized adjustments based on the patient's risk of rejection and their response to the treatment. This tailored approach ensured that each patient received the optimum level and type of immunosuppression, thereby increasing the likelihood of successful graft survival. The effectiveness of the treatment regimen implemented in the experimental group is corroborated by several previous studies. The administration of oral prednisolone has been shown to significantly reduce inflammation and modulate immune responses in various autoimmune and inflammatory conditions, including rheumatoid arthritis, and autoimmune hepatitis[17,18]. Similarly, the utilization of cyclosporine capsules has been extensively documented to exhibit remarkable immunosuppressive properties, effectively mitigating the risk of organ transplant rejection[19,20]. In addition, the remarkable application of tacrolimus eye drops, a highly potent

topical calcineurin inhibitor, has exhibited promising outcomes in diminishing the incidence of corneal graft rejection across various animal models and clinical investigations[21,22]. The use of prednisolone eye drops has also shown its value in providing local immunosuppression in the ocular environment, enhancing graft survival after corneal transplantation[23]. Thus, these pieces of evidence significantly support the experimental group's treatment regimen's effectiveness in the context of corneal transplantation. Overall, the experimental group's regimen demonstrated a more adaptive, comprehensive and personalized approach to immunosuppression in corneal transplantation, especially for high-risk patients

Moreover, monitoring immune markers like the CD4+/CD8+T cell ratio and interleukin levels, has been advocated as a valuable instrument for directing the adjustment of immunosuppressive treatment, aligning with the individual needs of each patient[24,25]. The reduction in the incidence of rejection episodes may be due to the more proactive and targeted approach to immunosuppression adopted in the study group. By monitoring immune markers, such as the CD4+/CD8+T cell ratio and interleukin levels, it was feasible to adjust the immunosuppressive regimen based on individual patient needs. Using this personalized approach, it was feasible to intervene early and effectively in cases where the risk of rejection was high, thereby reducing the overall incidence of rejection episodes. Monitoring these markers allows for a more personalized approach to immunosuppression, which can lead to better outcomes and a reduced risk of graft rejection[26,27].

The present study also demonstrated significant improvements in visual acuity outcomes for patients in the study group. This can be partly explained by the reduction in rejection episodes, as graft rejection is a major cause of vision loss following corneal transplantation. Additionally, the optimized immunosuppressive regimen may have led to better overall graft health and function, resulting in improved visual outcomes for the study group patients. The safety profile of the optimized immunosuppressive regimen was comparable to that of the standard regimen, suggesting that the novel regimen did not introduce any significant additional risks. This is an important finding, as it indicates that the improved efficacy of the optimized regimen does not come at the expense of patient safety.

SIGNIFICANCE STATEMENT

This study provides compelling evidence regarding the utilization of a staged optimized immunosuppressive regimen in high-risk corneal transplant patients. By comparing this novel approach with a conventional regimen, the study highlights its superior efficacy in reducing the rejection rate, prolonging the time to the first rejection episode and improving the graft survival rate. Moreover, the staged optimized immunosuppressive regimen demonstrated a comparable safety profile to the conventional regimen, reassuring clinicians of its suitability. Additionally, the treatment group experienced significant improvements in visual acuity, emphasizing the positive impact of corneal transplantation on vision. These findings have important clinical implications, offering a promising alternative for managing high-risk corneal transplant patients and suggesting potential advancements in enhancing patient outcomes and post-transplant success rates.

CONCLUSION

This study demonstrated that the optimized immunosuppressive regimen is a promising approach for managing patients undergoing corneal transplantation, particularly those at high risk for graft rejection. The personalized, risk-stratified strategy significantly improved graft survival, reduced the incidence of rejection episodes and led to better visual outcomes, without compromising patient safety. Future research should focus on validating these findings in larger, multicenter studies and exploring additional strategies to further optimize immunosuppressive regimens in corneal transplantation.

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