Topical Cyclosporine A in Corneal Graft Rejection

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Abstract: Corneal graft rejection is now the most common cause of graft failure after penetrating keratoplasty. This study was designed to determine whether, addition of 2% topical cyclosporine (CSA) to local and systemic steroids in treatment of endothelial corneal allograft rejection, would improve the outcome. A prospective randomized treatment trial was carried out on 40 consecutive corneal graft recipients, presenting with the first episode of endothelial graft rejection in two groups. Group one (20 patients) received topical steroids eye drops and systemic prednisolone (1 mg/kg) by oral route plus placebo. Group two (20 patients) received the same topical and systemic steroid therapy plus 2% cyclosporine A (CSA) eye drop. The patients were followed up for three months and their clinical outcomes were evaluated by the rates and time of rejection reversal. In group one, 14 (70%) cases had total reversal of graft rejection episode but in CSA group, it occurred in 18 (90%) cases (P=0.21) .Improvement were started within a mean period of 3 and 1.5 days respectively (P value<0.001). Among patients who sought treatment early (<6 days), the survival rates were 85% and 100% respectively (P=0.2). In high risk patients the rejection reversal rate was 66% in CSA group and 25% in the control group (P=0.5). Our study indicates addition of 2% CSA eye drop to topical and systemic steroids in graft rejection decreases the interval between treatment intervention and improvement of clinical signs. In high risk patients it may improve the reversal rate, however it needs further studies.

Key words: Cyclosporine A, Graft rejection, Penetrating keratoplasty

INTRODUCTION

Much of the success in penetrating keratoplasty (PKP) is due to improved surgical techniques and better donor tissue management. Improved medical management of corneal allograft rejection has produced survival rates of up to 95% in some reported series of low risk patients [1].

Corneal graft rejection is now the most common cause of graft failure after PKP [2,3]. The endothelium is the most important layer to be affected due to its role in maintaining clarity. Endothelial cells are lost at the time of surgery and for the first 2 to 3 years after surgery, as cells migrate to replace lost cells at the edge of graft[4]. A rejection episode results in loss of large numbers of endothelial cells and even if rejection process is reversed, sufficient number of cells may not survive to maintain graft clarity[5].Therefore, to maintain graft clarity, rejection episodes should be reversed as quickly as possible to preserve the maximum number of endothelial cells[6]. Reported rates of corneal graft rejection is between 3.5% to 65% according to the extent of recipient corneal vascularization [3,7].

Corticosteroids by topical, periocular, or systemic administration remained the main stay in the treatment and prevention of corneal graft rejection. Although some authors use only topical corticosteroid drops[8,9], others treat the more severe rejection episodes involving the endothelium with systemic and or subconjuctival steroids[1,10,11].

Because of complications of long term use of steroids more specific and less toxic agents are needed in the management of corneal graft rejection.

Systemic cyclosporine A has been useful in suppressing graft rejection after organ transplantation [12] .Cyclosporine A (CSA) is a fungal metabolite which represents a relatively new generation of specific immunosuppressive agents and selectively interferes with immune competent cells without causing generalized cytotoxic effects. Structurally, CSA is a hydrophobic, cyclic endecapeptide derived from the fungus Tolypocladium inflatumgans. It works mainly on T cells by binding to an intracellular peptide known as cyclophilin. Cyclophilin is a type of regulatory protein known as immunophilin that seems to control the synthesis of proteins involved in $CD4^+$ and $CD8^+$ T cell activation. It mediates its immunosuppressive effects by inhibition of calcineurin activity. CSA blocks the transcription and production of IL-2 and interferon-γ. It also inhibits the expression of high-affinity IL-2 receptors [13].

Cyclosporine has also been used in the treatment of corneal graft rejection [9,14] . As systemic

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CSA treatment induces systemic adverse effects such as hepatic and renal damage[15-17], this study was conducted to determine whether addition of topical cyclosporine to topical and systemic steroid regimen in the corneal graft rejection yielded superior outcomes in the rates and time of the graft rejection reversal.

MATERIALS AND METHODS

We conducted this respective study on 40 patients in two groups each with 20 patients with the first episode of endothelial graft rejection, managing at Shiraz University of Medical Sciences (Poostchi Ophthalmology Research Center). The clinical diagnoses of patients in both groups were shown in table 1.

Table 1: Original diagnosis

	Group1	*Group2*
Keratoconus	1	3
Aphakic bullous keratopathy	2	1
Pseudophakic bullous keratopathy	5	2
Herpes simplex scaring	3	4
Fuch's endothelial dystrophy	1	2
Re-graft	1	1
Corneal scar	6	5
Congenital hereditary endothelial dystrophy	1	2
Total	20	20

The diagnostic criteria for endothelial graft rejection was when an eye with the previously clear and thin graft became inflamed with cell and flare in the anterior chamber, kerato precipitate limited to the donor endothelium, thickening of the graft either diffusely (probable rejection), or in the form of advancing rejection line (definite rejection) and ciliary and conjunctival congestion. Excluded patients were those who presented only with epithelial or stromal rejection and the recipients of tectonic grafts.

The trial protocol was approved by the University Ethics Committee and informed written consent was provided from all participants. All patients were informed about the potential adverse effects of topical cyclosporine.

Complete ocular examination including checking of visual acuity by Snellen chart and slit-lamp evaluation was done .Ultrasound central corneal pachymetry was performed for each patient at the time of presentation and last follow up visit. The intervals between first symptom and intervention as well as treatment to clinical improvement were recorded for all patients.

Preparation of cyclosporine eye drops and placebo:
A clinic staff who was unaware of the study goals was trained to prepare the drug and placebo by dilution of intravenous 5% cyclosporine A vial (Novartis Pharma AG, Basle, Switzerland) with artificial tear (Sno-tear, Chauvin Pharmaceutical Ltd, England) to achieve 2% topical concentration or use only artificial tear to fill the similar 10 ml containers according to the patient's individual code. In this manner, the examiner and the patient were unaware of drug used. Patients were instructed to refrigerate the bottle of drug, shake it before use and replace it with a new bottle after one month.

Treatment intervention: 40 consecutive patients were enrolled in the study and allocated by balanced block randomization into two groups during 8 months period. Group one (20 patients) was treated with every hour 1% prednisolone acetate eye drop in combination with oral prednisolone (1mg/kg/day) and placebo every 3 hours . The second group was treated with the above steroid regimen plus 2% cyclosporine A eye drop every 3 hours. The oral medication and eye drops in both groups were tapered after one week. Oral prednisolone was completely withdrawn by 6 to 8 weeks. Topical prednisolone drop was continued in low dose (2-3 drop/day) after one week and gradually tapered till the maintenance dose of 1 drop per day achieved. The cyclosporine or placebo was tapered after one week to 4 times a day for one week and then gradually discontinued during next 5 weeks. In 8 weeks, all these topical medications were discontinued except in high risk cases in which one drop of prednisolone was continued every other day for the next 4 weeks. Eyes with history of herpes simplex keratitis were also treated with systemic antiviral medication (acyclovir, 400 mg, twice daily by oral route) for at least 4 weeks. Finally, the eyes with elevated intraocular pressure were treated with topical and/or systemic anti - glaucoma medication. Any adverse effects of drugs were recorded at each examination.

Statistical analysis: Statistical comparison of the results was performed using general linear model and Fisher Exact test by SPSS 11.5 software (Chicago, IL) and statistical analysis of data within each group was performed using repeated measure test. The P value of less than 0.05 was considered statistically significant.

Evaluation of outcome: Patients were examined every day after initiation of treatment for one week and weekly until one month and then scheduled follow up examinations were undertaken monthly for 3 months. Patient complaints and complete examinations at each visit were recorded. The compliance was assessed by asking the patient about exact usage of medications.

The primary outcome measure was the rates and time of the rejection episode reversal. The response to treatment was monitored by assessing the improvement in clinical signs (reduction of graft thickness, return of corneal clarity, restoration of visual

acuity, reduction and disappearance of keratic precipitate, improvement in anterior chamber cellular reaction and disappearance of endothelial rejection line). The rates of graft survival in patients who had early intervention (<6 days) were also evaluated. The choice of 6 days as an indication of early presentation was arbitrary and was selected as 6 days which was the mean time of patient's presentation.

RESULTS

The mean age of the patients were 59±17.4 (range of 22-80) and 50±21.6 (range of 16-80) years in group one and two respectively with no statistically significant difference .Also sex distribution between groups was nearly equal (F/M ratio of 0.66 and 0.81, respectively). Corneal vascularization (deep vessels in peripheral stroma, between 2-4 clock hours) was present in 4 cases in group one and 3 cases in group two. The mean interval between grafting and the rejection episodes were relatively equal in two groups (20±3.4 and 22±1.7 months respectively, P value=0.46). The time between the onset of rejection symptoms and intervention were also similar between groups (6.9 ±3.4 and 6±2.4 days respectively, P value=0.34)

Reversal of rejection episode: In group one, topical plus systemic steroids failed to reverse the rejection episode in 6 (30%) patients, compared with 2 (10%) cases in group two (local and systemic steroids plus 2% cyclosporine eye drop). However, the difference was not statistically significant (P=0.21).

In patients who sought treatment early (<6days), the survival rates were 85% in group one and 100% in group two (P value= 0.2) (Table 2).

Table 2. Clinical outcome in two groups

Successful Rejection Treatment	Group one	Group two	*p-value*
In high risk patients	1/4 (25%)	2/3 (66%)	0.2
In patients with early presentation	11/13(85%)	14/14(100%)	0.5
Overall	14/20 (70%)	18/20(90%)	0.21

The interval between treatment and clinical improvement was significantly shorter in CSA group (1.5±1 days) compared with placebo (3±1 days) (P value < 0.001). The mean intraocular pressure at presentation was 20±4.8 mmHg (range of 13-28 mmHg) in group one and 21.7±4.4 mmHg (range of 16-29 mmHg) in group two. The mean intraocular pressure at the end of study were 15.6±2.9 (range of 9-20mmHg) in group one and 16.6±2.2 mmHg (range of 14-21 mmHg) in group two with no statistically significant difference between groups at presentation and at the end of the study (P values=0.78 and 0.80 respectively)

Endothelial rejection line (Khodadoust line) was seen in 16% of patients in group one and 14% of patients in group two. Patients under topical CSA treatment tolerated the medication well and only two patients (10%) experienced severe ocular discomfort (burning sensation), though it didn't lead to discontinuation of topical CSA. The central corneal thickness measurements by ultrasound technique are shown in Table 3.There is no statistically significant difference between groups regarding two measured corneal thicknesses (P values of 0.50 and 0.83 at arrival and last follow up examinations respectively).

Table 3. Central corneal thickness (μm)

	At rejection time		Two months post rejection	
	Mean ±SD	Range	Mean ± SD	Range
Group one	630 ± 85	528-860	575 ± 78	497-855
Group two	639 ± 116	549-1084	574 ± 112	543-1013

DISCUSSION

Systemic administration of CSA is beneficial in graft survival, decrease in hospital stay and reduction of iatrogenic complications in organ transplantation. It has been used extensively to suppress rejection after renal[12] ,bone marrow[18], cardiac and liver transplantations [19]. Systemic cyclosporine has also been used to treat various autoimmune diseases such as ocular inflammatory diseases[20,21], psoriasis[22], rheumatoid arthritis[23], and myasthenia gravis[24]. It has been used systemically with some success in patients with various ocular manifestation of systemic immune diseases including Grave's ophthalmopathy[25], corneal peripheral melting syndrome[26], Behcet's disease[27], as well as intermediate and posterior uveitis unresponsive to conventional corticosteroids and cytotoxic therapy[28]. Unfortunately significant nephrotoxicity[15,16], hypertension[29], and hepatotoxicity[17] have been associated with its systemic use. Ocular side effects due to systemic use of CSA include decreased vision, lid erythema, non specific conjunctivitis, visual hallucination, and conjunctival and retinal hemorrhage secondary to anemia[30].

Several clinical studies have demonstrated the efficacy of CSA as a topical immunosuppressant in eye disorders. Application of topical 0.05% cyclosporine A, twice daily was successful in the treatment of dry eye patients[31]. In a study of 11 high risk keratoplasty patients treated with 2% topical CSA, 10 corneas (91%) remained clear for a mean follow up of 16 months period[32].

Holand et al [33] reported the use of topical CSA to treat 43 patients with a variety of anterior segment inflammatory conditions, including 11 high-risk keratoplasty patients for whom corticosteroid therapy failed. None of the 11 corneas were rejected during the 7 to 30 months treatment period. Mauro et al [34] compared the rejection free graft survival rates of 42 patients who underwent PKP and were treated with 2% CSA eye drop with 50 patients who underwent same procedure without administration of CSA . Twenty months after the surgery, the rejection free graft survival rate for the former group (92.7%) was significantly higher than the other group (88.6%). Kenji-Inoue et al[35] showed that the rate of free rejection survival for the CSA treated group (80.2%) was significantly higher than the control group (68.0%). In another study, Chen and colleagues[36] found that CSA when applied topically to the cornea could prevent the initiation of immune graft rejection and reverse a graft rejection in progress. Zhao and Jin[37] used 0.5% topical CSA to treat 16 patients with refractory corneal graft rejection and achieved a complete cure in nine eyes and significant improvement in another six eyes. To prevent the recurrence, they supported continuation of CSA for at least 12 months after reversal. In contrast, Zhang[38] found that the effect of 2% topical CSA was the same as that of the topical steroids in mean survival time and reducing the risk of allograft rejection in rats. In the other hand according to which observed by Price et al[14] topical cyclosporine 0.05%was effective in prevention of graft rejection in low risk patients as prednisolone acetate 1%. However this finding may be due to the low dose administration of Cyclosporine or early tapering of steroids.

In our study, the rejection reversal rate of 90% in CSA group and 70% in non CSA group were slightly higher than the studies in which reversal rates were between 50% and 75.9% [3,39] ; however, the difference was not statistically significant(P value =0.21). In contrast, we found that addition of CSA is significantly beneficial in shortening the duration of rejection reversal (P value=0.001). Moreover, none of the complications of systemic cyclosporine treatment were observed by this rout of administration.

The results in patients who presented early showed a higher survival rate in group two (100%) compared to group one (85%).Also in high risk patients addition of CSA seems to be more beneficial but due to paucity of these cases in both groups the difference was not statistically significant. Although the differences in survival and rejection reversal rates were also not statistically significant; we showed that the addition of cyclosporine would reverse the rejection episode faster and may preserve more endothelial cells. However, corneal thickness measurements had no statistically significant difference between groups and this claim needs further studies on endothelial cells to prove.

CONCLUSION

Addition of 2% CSA eye drop to topical and systemic steroids will improve the time to reversal of rejection episode. It may be especially useful in treatment of high risk patients. Although more well-designed randomized trials with larger sample sizes are needed to evaluate its long term safety and efficacy, topical CSA appears to be a well tolerated and effective ocular immunosuppressant.

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