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A Clinical Trial Comparing the Safety and Efficacy of Topical Tacrolimus versus Methylprednisolone in Ocular Graft-Versus-Host Disease

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Abstract

Purpose—To evaluate the safety and efficacy of topical tacrolimus 0.05% vs. topical methylprednisolone 0.5% in patients with ocular graft-versus-host disease (GVHD).

Design—Phase I/II, prospective, randomized, double-masked clinical trial.

Subjects—Eighty eyes from 40 patients diagnosed with chronic ocular GVHD were enrolled.

Methods—Forty patients with ocular GVHD were randomized; 24 patients were treated with topical tacrolimus 0.05% and 16 patients with topical methylprednisolone 0.5% twice a day for 10 weeks, in addition to continuing their baseline treatment regimen.

Main Outcome Measures—Safety was evaluated based on occurrence of adverse events. Tolerability was assessed based on subjects' reports of discomfort after drop instillation.

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Intraocular pressure (IOP) was monitored. The main efficacy endpoints were: corneal fluorescein staining (CFS), tear break-up time (TBUT), Schirmer test, and expression of the ocular surface inflammatory markers human leukocyte antigen-DR (HLA-DR) and intercellular adhesion molecule-1 (ICAM-1). Symptoms were evaluated using the Ocular Surface Disease Index (OSDI).

Results—After 10 weeks of treatment, no major adverse events occurred in either treatment group, and there was no significant difference in the composite tolerability scores between the two groups (P=0.06). However, burning sensation was more pronounced with tacrolimus (P=0.002). Topical tacrolimus was more effective than methylprednisolone in reducing the CFS score at week 10 (55% vs. 23% reduction, respectively; P=0.01), and achieved significant improvement in TBUT when compared to baseline (P<0.001). OSDI score reduction achieved statistical significance with tacrolimus (27% reduction; P=0.02) but was marginal with methylprednisolone (32% reduction; P=0.06). ICAM-1 expression by ocular surface epithelium decreased significantly in both groups (tacrolimus P=0.003; methylprednisolone P=0.008), while HLA-DR expression significantly decreased only in the tacrolimus group (P=0.03). Schirmer test scores did not change significantly in either group during the study; IOP increased significantly with methylprednisolone at week 10 (P=0.04).

Conclusions—Topical tacrolimus 0.05% is safe, generally well tolerated and effective for the treatment of ocular GVHD, without the hypertensive effects of topical corticosteroids.

Introduction

Hematopoietic stem cell transplantation (HSTC) is a potentially curative treatment for a variety of hematological disorders.[1] The accessibility and improvements in the techniques of this therapeutic procedure have increased patients survival; however, long term complications can impair the quality of life in these patients.[1] A common complication of HSTC is graft-versus-host disease (GVHD), an inflammatory response derived from donor cell infiltration and directed against host tissues. Chronic GVHD causes serious morbidity and mortality in patients undergoing HSCT, and the majority of patients with chronic GVHD present ocular involvement.[1,2] Dry eye is the most common ocular finding of the disease, and is present in almost 90% of the cases. Other ocular findings in patients with chronic GVHD include conjunctival hyperemia, dysfunction of the lacrimal and meibomian glands, corneal ulceration and perforation, pseudomembranous conjunctivitis, ocular surface scarring, among others. The posterior segment of the eye is not affected with the same frequency than the ocular surface; however, it is involved in about 12.8% of cases of ocular GVHD.[1,2]

Treatment of systemic manifestations of chronic GVHD is primarily based on systemic medications, such as corticosteroids and a variety of immunosuppressants.[1,2] Although systemic treatment can have positive effect on the ocular surface, it is often not sufficient to control the ocular disease.[1] Topical medications play a critical role in the treatment of ocular GVHD, contributing to reduction of local symptoms and inflammation. Among available local treatment options include topical corticosteroids, cyclosporine, autologous serum, lubricant eye drops and ointments, and lacrimal punctal occlusion among others.[1] Despite these, adequate control of ocular surface inflammation and patient symptoms in ocular GVHD often remains a challenge.

Tacrolimus is an immunomodulatory agent that impairs T cell activity via inhibition of the calcineurin enzyme, and is reported to be 10 to 100 times more potent than cyclosporine A, the only drug currently approved for the treatment of dry eye in the United States.[3] Tacrolimus was initially approved as a systemic immunosuppressant in liver transplantation; currently it is used as immunosuppressant in different organ transplants and as a modulator in various inflammatory diseases, including systemic GVHD.[4,5] In ophthalmology, efficacy of treatment with topical tacrolimus has been reported in severe allergic conjunctivitis, Sjögren's syndrome, prophylaxis after corneal transplantation, among other patologies.[6–11]

Numerous cytokines and biomarkers are expressed by the ocular surface during inflammation.[12–14] Specific surface markers, such as human leukocyte antigen-DR (HLA-DR), a main component of the major histocompatibility complex II, and the intercellular adhesion molecule-1 (ICAM-1 [CD 54]), have been shown to be significantly upregulated in patients with dry eyes secondary to ocular GVHD.[12–14] In this regard, impression cytology of the conjunctival epithelium has shown utility and consistency assessing changes in the expression of HLA-DR and ICAM-1 at the ocular surface in response to anti-inflammatory treatment.[14–18]

The aim of this randomized trial was to evaluate the safety and efficacy of topical tacrolimus 0.05% compared to topical methylprednisolone 0.5%, a drug with proven anti-inflammatory effects, in reducing the signs and symptoms in patients with chronic ocular graft-versus-host disease.[19,20] A few retrospective and non-comparative open-label studies have also reported improvement of signs and symptoms of ocular GVHD after treatment with topical tacrolimus 0.02% and 0.03%,[21–23] but this is the first randomized, controlled, double-masked prospective trial investigating the safety and efficacy of topical tacrolimus for the treatment of ocular GVHD.

Materials and Methods

Study design and participants

We conducted a prospective, randomized, double-masked, phase I/II, clinical trial to evaluate the safety and efficacy of topical tacrolimus 0.05% compared to topical methylprednisolone sodium succinate 0.5%, twice a day for 10 weeks in patients with refractory ocular GVHD. The study drug was added to the patients' baseline treatment regimen, and patients were evaluated at baseline, week 5 and week 10. This study was approved by the Massachusetts Eye and Ear Infirmary (MEEI) institutional review board, by the US Food and Drug Administration (IND application #119462), followed the principles of the Declaration of Helsinki, complied with the HIPPA privacy rule, and was registered at www.clinicaltrials.gov.

All participants were 18 years or older, with a diagnosis of chronic ocular GVHD according to the National Institutes of Health diagnostic criteria, had a corneal fluorescein staining score (CFS) equal or higher than 4 in either eye [National Eye Institute (NEI) grading system],[24] and at least moderate dry eye symptoms (score ≥ 23) according to the Ocular Surface Disease Index (OSDI).[25] We excluded patients with: 1) history of immune diseases other than GVHD, herpetic keratitis or ocular malignancy; 2) treatment regimen changes

with topical cyclosporine, autologous serum, anakinra, or oral tetracycline compounds within 30 days prior to enrollment; 3) treatment regimen changes with systemic immunosuppressants or topical anti-glaucoma medications within 15 days prior to enrollment; 4) signs of infection or a corneal epithelial defect larger than 1 mm^2; 5) current use of topical steroids more than twice a day; 6) ocular surgery within 3 months or contact lens use within 2 weeks prior to enrollment; 7) pregnant or lactating women; 8) participation in other interventional studies or any condition precluding patients from complying with the study requirements. In order to avoid exposing patients at risk of ocular hypertension as a result of corticosteroid therapy, patients (n=8) with a history of intraocular pressure higher than 22 mmHg in either eye or known personal or family history of glaucoma in first degree relatives were allocated by the MEEI pharmacy to the tacrolimus arm. Subjects who met all the inclusion/exclusion criteria (n=32) were randomized in a 1:1 ratio to the tacrolimus and methylprednisolone treatment arms, respectively (Figure 1). Randomization was performed by the MEEI pharmacy using a random code generator based on a permuted-block design (Research Randomizer v4.0; http://randomizer.org).

Outcome measures

Safety—We evaluated the occurrence of local and systemic adverse events with both treatments. Tolerability was evaluated at every visit with a self-response questionnaire that assessed burning sensation, discharge, redness, itchiness, and foreign body sensation in a scale from 0 to 4 (none 0, trace 1, mild 2, moderate 3 and severe 4). Additionally, intraocular pressure and best-corrected visual acuity were assessed in all visits.

Efficacy—We evaluated the following efficacy endpoints: symptoms (OSDI score), corneal fluorescein staining, tear break-up time (TBUT), Schirmer test score, and the expression of HLA-DR and ICAM-1 on the ocular surface (conjunctival impression cytology).

Self-reported symptoms of dry eye were evaluated according to the OSDI questionnaire protocol and before performing any procedures during the study visits.[25] TBUT was assessed after instillation of 15μL of sterile fluorescein solution (FLUCAINE; Ocusoft, Inc., TX) in the inferior fornix. Corneal epithelial staining was assessed 2 minutes after instillation of fluorescein using a slit-lamp and cobalt blue illumination, according to the NEI grading system.[24] Schirmer test type I with anesthesia was performed using a 5 × 35 mm strip of Whatman #41 filter paper placed in the inferotemporal cul-de-sac for 5 minutes. Impression cytology specimens were obtained from the superotemporal conjunctiva of the right eye at baseline and week-10 visits (procedure detailed below). In all cases clinical assessment of the ocular surface was performed following the same sequence as described above.

Impression cytology and flow cytometry analysis—Impression cytology was obtained at the end of the baseline and week-10 visits. Topical anesthesia (0.5% proparacaine hydrochloride, Alcaine; Alcon Inc., TX) was applied into the right eye (at least 15 minutes after fluorescein instillation), and then a disc of polyethersulfone filter paper (0.2 micron pore, 13 mm diameter [Supor 200 Membrane, Pall Co., NY]) was placed onto the

superotemporal bulbar conjunctiva and gently pressed for 10 seconds. Samples were stored immediately at 4°C in tubes containing 4 ml of cell culture medium (RPMI 1640, Lonza, MD) with 10% of fetal bovine serum (GIBCO Invitrogen, New Zealand). Cells were separated from the filters within a maximum of 3 hours after collection by agitation for 15 minutes, and centrifuged (1200 rpm, 5 minutes, 4°C). The cells extracted from the filter paper were incubated with Fc receptor block (eBioscience) for 20 minutes, then stained with fluorescent-conjugated antibodies or an isotype control for one hour, and finally fixed (Leinco Techonologies Inc., MO). The following antibodies and negative controls were used: PE/Cy7 conjugated anti-human HLA-DR and PE/Cy7 conjugated mouse IgG2a isotype control, and Pacific Blue™ conjugated anti-human CD54 (ICAM-1) and Pacific Blue™ conjugated mouse IgG1 isotype control (BioLegend, San Diego, CA). Flow cytometry analysis was performed on a BD LSR II flow cytometer (BD Biosciences, San Jose, CA) and data analysis was executed using Summit v4.3 software (Dako North America, Inc., CA). For each patient and visit the respective isotype controls were used to determine the frequency of positive cells for each target.

Study treatment—The study drugs were formulated under sterile conditions, assigned, and distributed to patients by the Massachusetts Eye and Ear Infirmary pharmacy. Topical tacrolimus 0.05% was compounded as a suspension from commercially available tacrolimus solution for intravenous use (Prograf, Astellas Pharma US, Inc., IL). Topical methylprednisolone 0.5% was compounded as a suspension from commercially available methylprednisolone sodium succinate dry powder for intravenous use (Solu-Medrol, Pfizer, Inc., NY). Since according to the fabricant methylprednisolone is soluble in alcohol-based vehicles, and tacrolimus can also be formulated in the same type of vehicle,[9–11] both study medications were compounded with polyvinyl alcohol 1.4% sterile ophthalmic solution (LiquiTears, Major Pharmaceuticals, Inc., CA) as vehicle, thus avoiding an additional variable in the treatment regimens.

Statistical analysis—We estimated a sample size of 40 participants with a study power of 90% assuming that 60% of the subjects would be randomized to the tacrolimus 0.05% arm and 40% to the methylprednisolone 0.5% arm. We constructed a conservative estimate of the study power using a t-test comparison of the predicted distribution of CFS (the main objective outcome), and for which some data was available on the use of topical tacrolimus at a similar concentration to treat GVHD-associated dry eye.[22] Safety variables were analyzed with respect to the treatment that participants actually received. Efficacy variables were evaluated using an intent-to-treat analysis. We used each participant's randomized treatment assignment to classify treatment groups and outcomes. We averaged the scores from both eyes for all measured variables, with the exception of OSDI where only one score per subject was obtained. We evaluated the change of all variables from baseline to each follow-up visit, and monitored for baseline imbalances between both treatment groups. To evaluate the effect of each treatment we analyzed the score change between baseline and follow up visits in each group and also compared these changes between the two groups using the *t*-test for normally distributed variables and the Wilcoxon rank sum test for non-normally distributed variables. A two-sided *P* value of less than 0.05 was considered statistically significant.

Results

Forty patients, 25 males and 15 females, with a mean age of 56 years were enrolled and randomized to the two treatment arms. A total of 24 and 16 subjects were assigned to the tacrolimus and methylprednisolone groups, respectively (Fig. 1). The baseline demographics and ocular characteristics of the participants are shown in Tables 1 and 2.

Safety Endpoints

Tacrolimus 0.05% and methylprednisolone 0.5% were safe and there were no reports of serious local or systemic adverse events related to the study treatments. Seven patients did not complete all the follow-up visits, where: three patients were lost to follow-up in the tacrolimus group, three patients decided to withdrew due to burning sensation when applying topical tacrolimus, and one patient from the topical methylprednisolone group was withdrawn after presenting a geographic corneal epithelial defect. The epithelial defect completely healed after appropriate treatment was established. Efficacy outcomes in these patients were included based on the intent-to-treat and last observation carry-forward analysis.

To assess tolerability, a drug-induced discomfort composite score (0 to 20) was calculated for each patient by adding the scores of burning sensation, discharge, redness, itchiness, and foreign body sensation (scale, 0 to 4 for each variable) referred by patients after instillation of the treatment drops (Fig. 2). The mean discomfort scores were 11.2 and 7.9 at week 5, and 12.2 and 8 at week 10 for topical tacrolimus and methylprednisolone, respectively. There was no significant difference between the mean composite scores in the two groups (week 5, P=0.05; week 10, P=0.06) (Fig. 2A). When analyzed individually, the burning sensation score was higher in the tacrolimus than in the methylprednisolone group, 3.6 and 1.6 at week 5 (P<0.001), and 3.5 and 2.2 at week 10 (P=0.002), respectively. Burning sensation was reported as severe (score of 4) by 75% of the subjects in the tacrolimus group and by 66% in the methylprednisolone group. Most patients reported the burning sensation to last no more than two minutes after drop instillation; however, three patients withdrew from the study due to intolerance (Fig. 2B).

The mean intraocular pressure did not change in the group treated with tacrolimus (week 5, P=0.50; week 10, P=0.20). In the group treated with methylprednisolone the IOP increased at week 5 (P=0.04) and week 10 (P=0.04) when compared to baseline, but this increase was not statistically significant when compared to tacrolimus (week 5, P=0.23; week 10, P=0.67). The mean IOP at baseline, week 5 and week 10 in the tacrolimus group was 15.6, 16 and 16.5 mmHg, while in the methylprednisolone group was 15.3, 17.5 and 17 mmHg, respectively (Fig. 2C).

Efficacy Endpoints

Treatment with topical tacrolimus was more effective reducing corneal fluorescein staining than methylprednisolone after 10 weeks of treatment (P=0.01) (Fig. 3; Table 2). Tacrolimus significantly reduced the CFS score from 8.2 at baseline to 5.6 (32% reduction; P=0.02) after 5 weeks, and to 3.7 by week 10 (55% reduction; P<0.001). Treatment with topical

methylprednisolone significantly reduced the mean corneal fluorescein staining score from 8.6 at baseline to 5.9 (31% reduction; P<0.001) at week 5, and to 6.6 by week 10 (23% reduction; P=0.09).

Tacrolimus reduced the OSDI baseline score from 58 units at baseline to 52 units (10% reduction; P=0.35) after 5 weeks, and 42 units after 10 weeks (27% reduction; P<0.02) (Fig. 4B). Methylprednisolone reduced the OSDI baseline score from 41 to 30 units (27% reduction; P=0.004) after 5 weeks, and 28 units by week 10 (32% reduction; P=0.06) (Fig. 4A). Despite randomization, significant disparities in OSDI scores at baseline (P=0.02) prevented from direct comparisons of OSDI change between the two treatments (Fig. 4; Table 2).

Treatment with tacrolimus increased the mean TBUT from 0.7 seconds at baseline to 1.4 seconds after 5 weeks of treatment (P=0.49), and to 2.6 seconds after 10 weeks (P=0.003). Methylprednisolone increased the mean TBUT from 0.6 at baseline to 1.1 seconds at week 5 (P=0.09) and 1.0 second by week 10 (P=0.42). There was no statistically significant difference in TBUT changes between the two treatment groups after 10 weeks (P=0.06) (Fig. 5; Table 2).

Clinically, both treatments improved mean visual acuity after 10 weeks. Tacrolimus treatment increased mean best-corrected visual acuity from LogMAR 0.19 at baseline to 0.13 at week 10 (P=0.12). Methylprednisolone significantly improved visual acuity from LogMAR 0.26 to 0.13 (P=0.04). When changes between the two groups were compared there was not statistically significant difference (P=0.56) (Table 2). Schirmer test scores did not change significantly in either of the two groups after 10 weeks of treatment (Table 2).

Flow Cytometric Analysis

To assess ocular surface inflammation we determined HLA-DR and ICAM-1 positive cells at baseline and 10 weeks after treatment. When compared to baseline, patients in both treatment groups achieved a statistically significant reduction in the frequencies of HLA-DR and ICAM-1 positive cells after 10 weeks. Tacrolimus significantly reduced the frequencies of HLA-DR positive cells from 8.7% at baseline to 4.7% at week 10 (46% reduction; P=0.03), while in the methylprednisolone group the frequencies of HLA-DR positive cells decreased from 9.5% at baseline to 7.2% at week 10 (24% reduction; P=0.09). The frequencies of ICAM-1 positive cells were significantly reduced after treatment with tacrolimus, from 40.9% at baseline to 24.8% at week 10 (39% reduction; P=0.003); methylprednisolone induced a change in ICAM-1 positive cells from 52.9% at baseline to 31.6% at week 10 (40% reduction; P=0.008) (Fig. 6).

Discussion

This study demonstrates that treatment with topical tacrolimus 0.05% is safe and does not cause serious adverse effects in patients with chronic ocular GVHD. Furthermore, our data suggest that topical tacrolimus 0.05% twice a day for 10 weeks is as effective as topical methylprednisolone 0.5% in reducing clinical signs and symptoms of dry eye in these patients. Tacrolimus has been used at different concentrations, from 0.005% to 0.1%, and

compounded with different vehicles, such as olive oil and balanced salt solution, and rendered mixed results in the treatment of ocular surface diseases.[6–11,22] To date, there is no consensus regarding the optimal concentration or vehicle to formulate topical tacrolimus. Experimental data in animals have shown that tacrolimus 0.05% achieved high levels of the drug in the aqueous humor and especially in the cornea after topical application, while being safe.[26] Additionally, clinical studies have reported safe use of topical tacrolimus at higher concentrations (0.1%) for the treatment of allergic conjunctivitis.[9,10] Based on this evidence, the severity of the ocular condition in our cohort, and the lack of response to other treatments, we decided to formulate tacrolimus at a concentration of 0.05%, a higher concentration than used in other studies for ocular GVHD and dry eyes, looking at the same time for safety and greater anti-inflammatory effect. To our understanding, this is the first clinical study to use topical tacrolimus in such concentration. We selected polyvinyl alcohol, which provides additional lubrication and has been used as a tear substitute, as a vehicle in an attempt to improve the therapeutic effect and tolerability of tacrolimus. Since we used tacrolimus in its soluble presentation for intravenous administration, polyvinyl alcohol provided a reasonable option as a vehicle for both tacrolimus and methylprednisolone.[9–11,26]

At the conclusion of this study, there was no significant difference regarding overall tolerability between the two treatments. However, more patients in the tacrolimus arm rated burning sensation as severe within the first minutes upon drug instillation; this effect was significantly more noticeable in the first month of treatment. Occurrence of local side effects and withdrawals due to topical tacrolimus intolerability in this study (12%) was significantly lower than those reported in other studies using similar concentrations of the drug.[6,9,11,22] In this regard, Reinhard et al. reported a withdrawal rate of 40% after a 3-month course of topical tacrolimus 0.06% in patients undergoing penetrating keratoplasty.[11] Meanwhile, Sanz-Marco et al. reported a 7% withdrawal rate over 3 months with tacrolimus 0.03% in patients with ocular GVHD.[13] In comparison, topical cyclosporine 0.05%, an FDA approved treatment for dry eye disease, has shown similar or higher rates of intolerance in various studies.[22,27,28] Sanz-Marco et al. reported that 48.27% of patients with ocular GVHD reported intolerance to topical cyclosporine 0.05%.[22] Given the gradually increased patient tolerance to tacrolimus instillation over the 10-week treatment course in this study, and its close association with significant improvement in corneal fluorescein staining, it is reasonable to believe that topical intolerability of tacrolimus in ocular GVHD is related to the degree of corneal epitheliopathy, which can be very severe especially at the outset of treatment. This may explain why patients with ocular GVHD also report significantly more intolerance to topical cyclosporine than patients with other types of dry eye.

In the present study, methylprednisolone 0.5% induced a mild increase of IOP in patients without risk factors for developing glaucoma although the magnitude of this increase was not considered clinically significant. The group treated with tacrolimus did not experience IOP changes, confirming previous reports in this regard.[9,10,21] Moreover, Jung et al. reported that IOP in patients with ocular GVHD significantly decreased and remained within normal range during a 12-month course of treatment with tacrolimus ointment 0.02%.[21]

The main finding of this study is that topical tacrolimus significantly reduced the symptoms of dry eye with sustained reduction of corneal fluorescein staining during the treatment

period. An explanation is that tacrolimus has a progressive effect impairing T-cell division, while additionally enhancing T-regulatory cells proliferation, which modulates the inflammatory response and promote the wound-healing process.[1, 29–32] The use of different scoring systems to assess CFS in published studies makes comparisons difficult. However, patients treated with tacrolimus in this study experienced more reduction in CFS than the reported in studies using tacrolimus 0.02% or 0.03%, or cyclosporine 0.05% or 1%, to treat ocular GVHD. Moreover, in the current study the effects of tacrolimus 0.05% were measured and compared in a controlled and randomized fashion to methylprednisolone 0.5%. Based on our findings, topical tacrolimus 0.05% in polyvinyl alcohol as vehicle represents an alternative for the treatment of corneal epitheliopathy in ocular GVHD.[6,21–23,33–35] In addition, significant improvement in tear film stability after treatment with tacrolimus 0.05% may relate to enhancement of goblet cells function, an effect that has been previously reported with the use of topical cyclosporine.[36,37]

A direct comparison between symptom changes in the two treatment groups was not possible given statistically significant differences in the mean OSDI scores at baseline between the 2 groups. However, the percentage in the OSDI score reduction after 10 weeks of treatment was comparable in both groups. In fact, tacrolimus 0.05% and methylprednisolone 0.5% reduced OSDI scores more than 9.9 units, which has been reported by Miller et al. as the minimal (average) change to be considered as clinically significant in patients with dry eye.[38] Furthermore, tacrolimus reduced the mean symptoms of dry eye by more than 13.4 units, reported as the minimal change to be considered as clinically significant in cases of severe dry eye. Results reported in the literature are variable regarding symptoms improvement after dry eye treatment with topical calcineurin inhibitors such as cyclosporine A or tacrolimus. Even less evidence is available for the use of these agents in patients with chronic ocular GVHD and refractory dry eye. In this regard, one open label, non-randomized, comparative study showed a reduction of only 3 and 4 OSDI units after 3 months of treatment with topical tacrolimus 0.03% and cyclosporine 0.05%, respectively, in patients with refractory ocular GVHD who had been treated previously with topical autologous serum, artificial tears and punctal occlusion.[22,36,39]

In the current study none of the two treatment groups experienced significant changes in the mean Schirmer scores. Although some studies have shown mild increase of tear production after topical treatment with tacrolimus, others have shown no change at all.[7,22,40] In the studies that report Schirmer score improvement, some differences with the current and other studies are present, including higher baseline Schirmer scores (>5 mm), different dry eye disease etiologies, or Schirmer assessment without anesthesia.[21,22] In these studies, with higher Schirmer scores at baseline, impairment of the lacrimal unit may have been less severe. The Schirmer test with anesthesia evaluates mainly basal tear secretion (accessory lacrimal glands), while a Schirmer test without anesthesia would mainly evaluate reflex tearing. Patients in the current study presented with chronic and aggressive ocular surface disease that included lower mean Schirmer scores than in other reports.

The frequencies of ICAM-1 positive epithelial cells obtained from the conjunctival epithelium were significantly reduced with both treatments after 10 weeks. However, only tacrolimus 0.05% significantly reduced the expression of HLA-DR, likely due to its specific

effect on T-cell activity. Previous studies have shown reduced expression of various inflammatory markers at the ocular surface after treatment for dry eye disease.[15,16] The frequencies of HLA-DR positive cells on conjunctival impression cytology samples were reduced after treatment with topical cyclosporine 0.05% and 0.1% for at least 3 months, and after oral supplementation with omega 3/6 fatty acids for 3 months.[15–18] Baudouin et. al. described a reduction of HLA-DR positive cells percentage of 36.7% after 3 months and 50.5% after 12 months of topical treatment with cyclosporine 0.05%, and a reduction of 27.5% and 40.8% with cyclosporine 0.1%.[17] In the present study tacrolimus 0.05% reduced HLA-DR positive cells by 46% and ICAM-1 positive cells by 39% after only 10 weeks of treatment in the setting of chronic, refractory ocular GVHD. This clinical trial not only demonstrates the strong effect of topical tacrolimus reducing the expression of inflammatory markers on the ocular surface of patients with ocular GVHD, but also is the first showing this effect of topical tacrolimus in any other condition of the ocular surface. Our results demonstrate that conjunctival impression cytology is a valuable technique to assess and follow-up ocular surface inflammation in chronic ocular GVHD.

This study was limited by the absence of a placebo arm, which in many dry eye trials is characterized by a topical lubricant; however, we felt that given the disease severity of patients in this trial, inclusion of a placebo arm was not ethically acceptable. Continuation of patients' current ocular surface treatments, besides the two studied drugs, may create the perception of limited interpretability of the results; however, in the context of refractory disease, the two studied drugs were considered an addition to the ongoing therapy. In that regard, and in order to obtain more interpretable results, we strictly limited enrollment to subjects without recent changes in their current medications who could benefit from additional treatment due to persistent disease, and who matched the inclusion criteria. Furthermore we did not allow changes or addition of any topical medications during the clinical trial.

In conclusion, treatment with topical tacrolimus 0.05% twice a day for 10 weeks is safe and effective in reducing ocular surface signs, symptoms, and inflammation in patients with chronic ocular GVHD, and can be a therapeutic option for treating severe dry eye disease without the known hypertensive effects of corticosteroids.

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Abbreviations

CFS	Corneal fluorescein staining
GVHD	Graft-versus-host disease
HIPPA	Health Insurance Portability and Accountability Act

HSTC	Hematopoietic stem cell transplantation
HLA-DR	Human leukocyte antigen-DR
ICAM-1	Intercellular adhesion molecule-1
IOP	Intraocular pressure
IND	Investigational New Drug
MEEI	Massachusetts Eye and Ear Infirmary
NEI	National Eye Institute
OSDI	Ocular Surface Disease Index
TBUT	Tear break-up time

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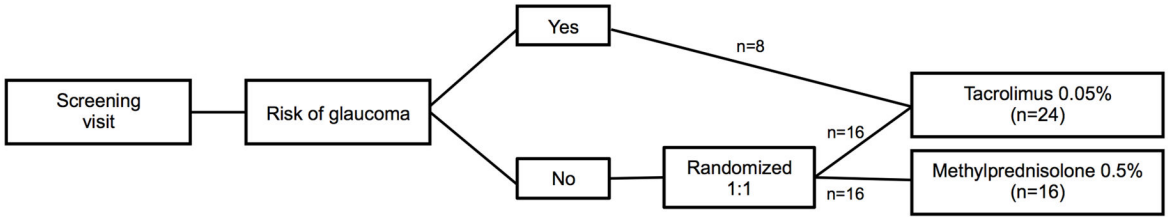

Figure 1.

Randomization scheme. Subjects who met the general inclusion/exclusion criteria (n=32) were randomized in a 1:1 ratio to the two treatment groups using a permuted-block design. Patients with history of intraocular pressure >22 mmHg or glaucoma in first-degree relatives (n=8) were allocated in the tacrolimus arm.

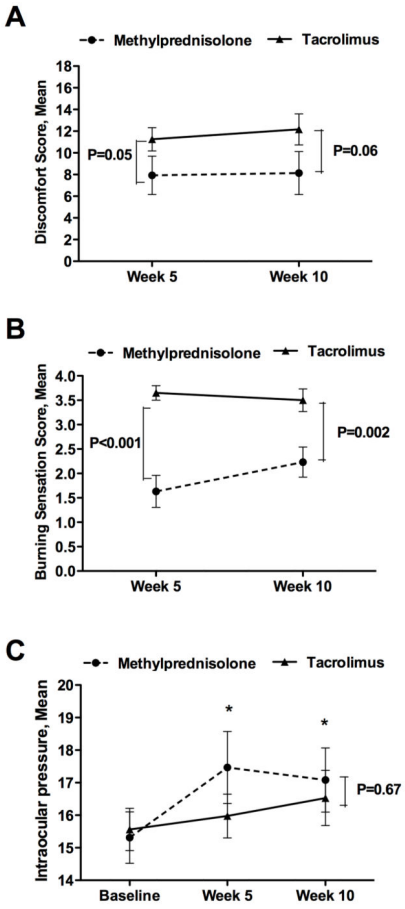

Figure 2.

Drug instillation-induced discomfort, burning sensation, and intraocular pressure induced by topical tacrolimus and methylprednisolone in patients with ocular GVHD. **(A)** Discomfort score, **(B)** burning sensation score, and **(C)** intraocular pressure after drug instillation. The mean intraocular pressure increased in patients treated with methylprednisolone at week 5 ($P = 0.04$) and week 10 ($P = 0.04$), when compared to baseline. The mean intraocular pressure did not change significantly in the patients treated with tacrolimus at week 5 ($P = 0.50$) or week 10 ($P = 0.20$). ***** $P < 0.05$ compared to baseline; GVHD, graft-versus-host disease.

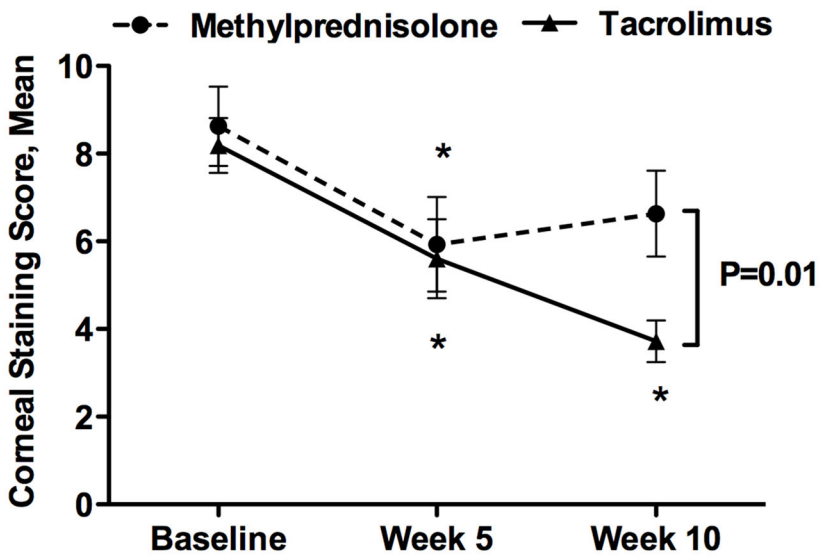

Figure 3.

Tacrolimus significantly reduced corneal fluorescein staining in patients with ocular GVHD at weeks 5 and 10 when compared to baseline ($P = 0.02$ and $P < 0.001$, respectively). Methylprednisolone significantly reduced corneal fluorescein scores at week 5 ($P = 0.02$) but not at week 10 ($P = 0.09$), when compared to baseline. Tacrolimus achieved more reduction in corneal fluorescein staining after 10 weeks when compared to methylprednisolone ($P = 0.01$). *****$P < 0.05$ compared to baseline; GVHD, graft-versus-host disease.

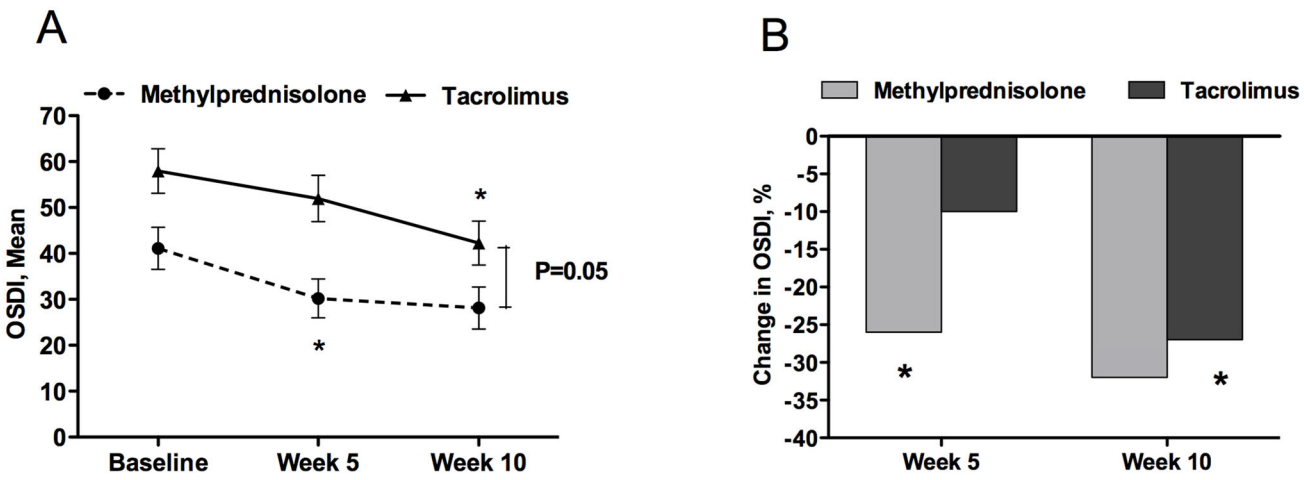

Figure 4.

(A) Tacrolimus decreased symptoms (OSDI) in patients with ocular GVHD at week 5 (P = 0.35) and week 10 (P = 0.02) when compared to baseline. Methylprednisolone decreased the OSDI score at week 5 (P = 0.004) and week 10 (P = 0.06) when compared to baseline. (**B**) Percentage change in the OSDI score at week 5 and week 10 compared to baseline. Tacrolimus reduced OSDI by 10% at week 5 (P = 0.35) and 27% at week 10 (P = 0.02). Methylprednisolone reduced OSDI by 27% at week 5 (P = 0.004) and 32% (P = 0.06) at week 10. OSDI, Ocular Surface Disease Index. *P < 0.05 compared to baseline; GVHD, graft-versus-host disease.

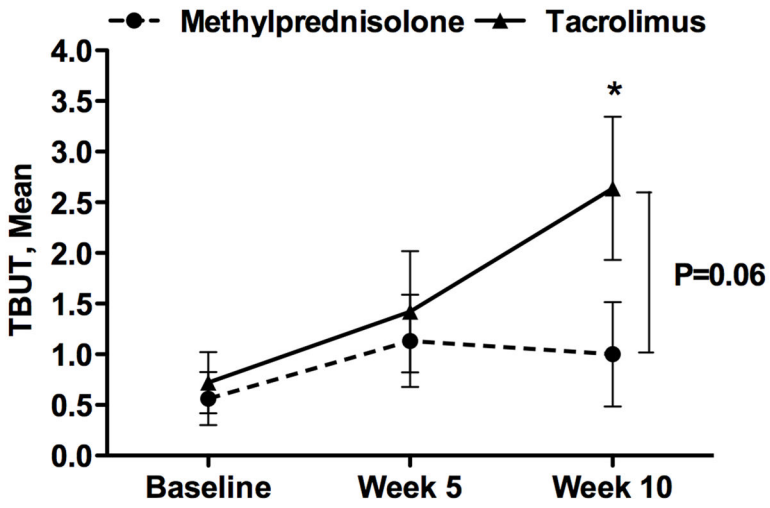

Figure 5.

Tacrolimus increased the mean TBUT after 5 weeks ($P = 0.49$) and 10 weeks ($P = 0.003$) in patients with ocular GVHD, when compared to baseline. Methylprednisolone did not affect TBUT at week 5 or 10 when compared to baseline ($P = 0.09$ and $P = 0.42$, respectively). TBUT, tear break-up time. *$P < 0.05$ compared to baseline; GVHD, graft-versus-host disease.

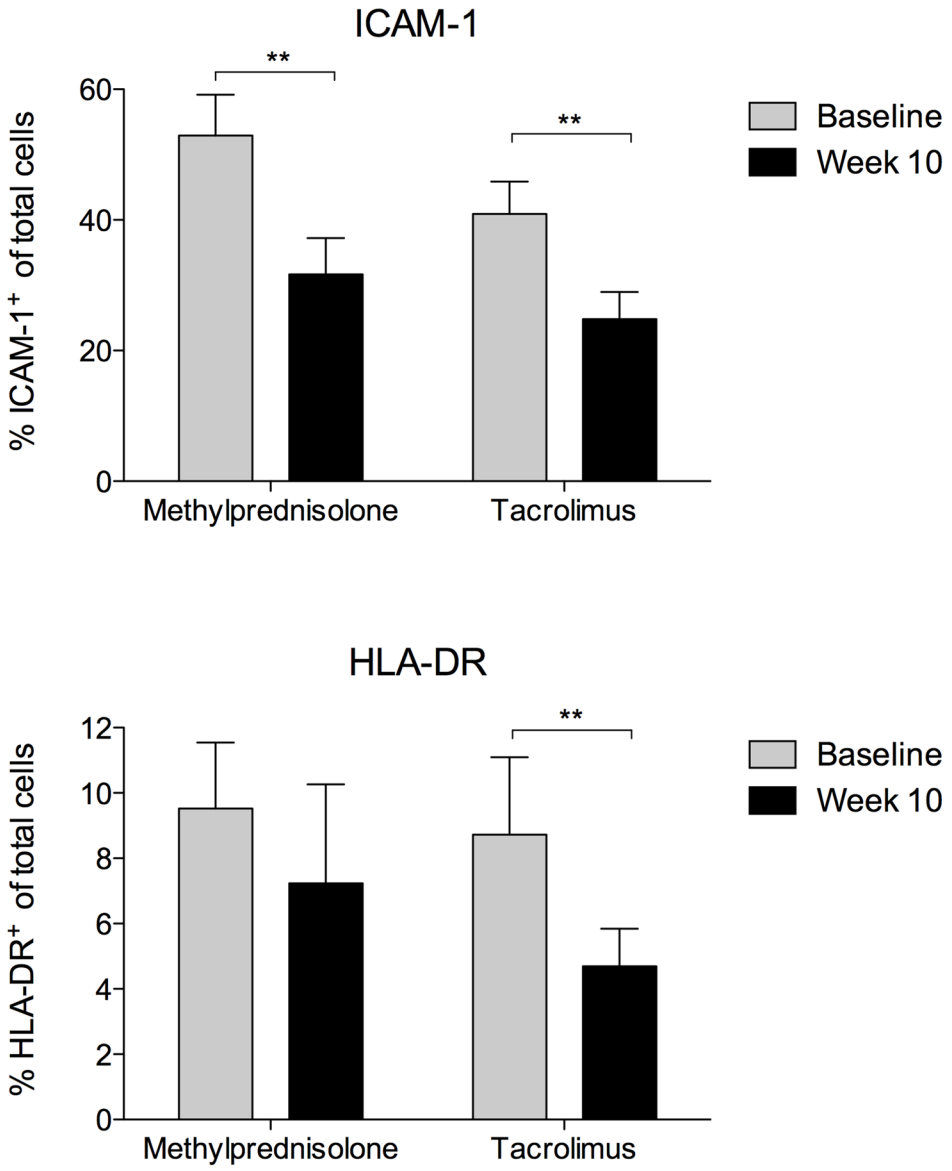

Figure 6.

Expression of inflammatory surface markers at baseline and week-10 in patients with ocular GVHD treated with topical tacrolimus and methylprednisolone. Topical tacrolimus and methylprednisolone significantly reduced the frequencies of ICAM-1 positive cells ($P = 0.003$ and $P = 0.008$, respectively). Only topical tacrolimus reduced significantly the frequencies of HLA-DR positive cells ($P = 0.03$). GVHD, graft-versus-host disease; HLA-DR, human leucocyte antigen-DR; ICAM-1, intercellular adhesion molecule-1.

Table 1

Demography and transplant details of patients enrolled in the study

Characteristics	Tacrolimus N=24 (%)	Methylprednisolone N=16 (%)
Age, mean ± SD	54 ± 12	58 ± 11
Gender, No.		
Male	16 (67)	9 (56)
Female	8 (33)	7 (44)
Primary disorder for which HSCT was performed		
Acute Myeloid Leukemia	5 (21)	7 (44)
Acute Lymphoid Leukemia	2 (8)	0 (0)
Chronic Myeloid Leukemia	2 (8)	1 (6)
Chronic Lymphoid Leukemia	1 (5)	0 (0)
Non-Hodgkin's Lymphoma	7 (29)	4 (25)
Hodgkin's Lymphoma	1 (5)	0 (0)
Myelodysplastic syndrome	2 (8)	3 (19)
Multiple myeloma	2 (8)	1 (6)
Thrombocytopenia	2 (8)	0 (0)
Pre transplant Conditioning regimen		
Total body irradiation + Chemotherapy	5 (21)	3 (19)
Chemotherapy alone	19 (79)	13 (81)
Ocular GVHD duration at baseline in months, mean ± SD	27 ± 34	28 ± 33

HSCT: hematopoietic stem cell transplantation, GVHD: Graft-versus-host disease, SD: Standard deviation.

Table 2

Outcome measures at baseline, week 5 and week 10

Outcome measures	Baseline		5 weeks		10 weeks	
	Tacrolimus (N= 24)	Methyl prednisolone (N= 16)	Tacrolimus (N= 20)	Methyl prednisolone (N= 15)	Tacrolimus (N= 18)	Methyl prednisolone (N= 15)
Ocular Surface Disease Index (OSDI)						
Mean (SD)	58 (24)	41 (18)	52 (23)	30 (16)	42 (20)	28 (18)
P value vs baseline	--	--	0.35	0.004	0.02	0.06
P value vs methylprednisolone	0.02	--	0.009	--	0.05	--
Corneal fluorescein staining (NEI grading system)						
Mean (SD)	8.2 (3.1)	8.6 (3.6)	5.6 (3.9)	5.9 (4.2)	3.7 (2.0)	6.6 (3.8)
P value vs baseline	--	--	0.02	<0.001	<0.001	0.09
P value vs methylprednisolone	0.68	--	0.81	--	0.01	--
Schirmer tear secretion score (mm)						
Mean (SD)	3.6 (3.2)	3.2 (3.3)	3.9 (2.8)	2.6 (1.4)	3.5 (2.3)	3.5 (2.8)
P value vs baseline	--	--	0.52	0.90	0.51	0.62
P value vs methylprednisolone	0.68	--	0.18	--	0.67	--
Fluorescein tear film break-up time (seconds)						
Mean (SD)	0.7 (1.5)	0.6 (1)	1.4 (2.6)	1.1 (1.8)	2.6 (3.0)	1.0 (2.0)
P value vs baseline	--	--	0.49	0.09	0.003	0.42
P value vs methylprednisolone	0.71	--	0.95	--	0.06	--
Visual acuity (logMAR)						
Mean (SD), LogMAR	0.19 (0.20)	0.26 (0.34)	0.13 (0.24)	0.20 (0.25)	0.13 (0.25)	0.13 (0.20)
P value vs baseline	--	--	0.02	0.96	0.12	0.04
P value vs methylprednisolone	0.43	--	0.13	--	0.56	--
Intraocular pressure (mmHg)						
Mean (SD)	15.6 (3.2)	15.3 (3.2)	16.1 (3.0)	17.5 (4.3)	16.5 (3.6)	17.1 (3.8)
P value vs baseline	--	--	0.50	0.04	0.20	0.04
P value vs methylprednisolone	0.80	--	0.23	--	0.67	--

SD, standard deviation; NEI, National Eye Institute; logMAR, logarithm of the minimum angle of resolution All the variables values are expressed as the average of both eyes, except for OSDI.