Original Research

Effects of Punctal Occlusion on Clinical Signs and Symptoms and on Tear Cytokine Levels in Patients with Dry Eye

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ABSTRACT Purpose: To investigate changes in signs, symptoms, and tear cytokines following punctal plug occlusion in patients with dry eye. Methods: A single-center study was conducted at Singapore Eye Research Institute. Nonabsorbable punctal plugs were inserted in the lower punctum of both eyes in patients with moderate dry eye. Over 3 weeks, in the more severe eye, dry eye symptoms, fluorescein corneal staining, Schirmer I (without topical anesthesia) test, tear film breakup time (TFBUT), and safety were assessed. Cytokine and matrix metalloproteinase-9 (MMP-9) levels in tear samples were measured. Results: Twenty-nine patients (mean age 49.8 years) with moderate dry eye were evaluated. At baseline, mean (standard deviation) global symptoms score was 53.8 (26.5), Schirmer I test score was 5.1 (2.8) mm, and TFBUT was 2.2 (0.6) seconds. After 3 weeks, punctal occlusion significantly reduced global irritation symptoms score (P < .001) and decreased fluorescein staining in all zones (P < .01) except the inferior zone (P = .42). No significant association between levels of cytokines or MMP-9 and either TFBUT or global irritation symptoms were observed at baseline. Levels of several cytokines and MMP-9 were higher in patients with Schirmer I test scores ≤ 8 mm at baseline. After 3 weeks of punctal occlusion, no significant changes in overall cytokine or MMP-9 levels were observed. Conclusions: Punctal plug occlusion provided symptomatic relief and reduced fluorescein staining in all except the inferior zone. However, insertion of punctal plugs had minimal effect on tear cytokines and MMP-9 levels, suggesting a need for earlier treatment with anti-inflammatory agents for management of dry eye disease.

KEY WORDS: cytokine, dry eye, matrix metalloproteinase, punctal plug, tear

Accepted for publication December 2015.

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This study was sponsored by Allergan plc, Dublin, Ireland. Writing and editorial assistance was provided to the authors by Kakuri Omari, PhD, of Evidence Scientific Solutions, Philadelphia, PA, USA, and funded by Allergan plc. All authors met the ICMJE authorship criteria. Neither honoria nor payments were made for authorship.

Disclosures: L. Tong has nothing to disclose; R. Beuerman has served as a consultant to Allergan plc, AB SCIEX, and Santen and holds patents in the field of research; S. Simonyi and D.A. Hollander are employees of Allergan plc; M.E. Stern was an employee of Allergan plc at the time the study was conducted.

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I. INTRODUCTION

The International Dry Eye WorkShop (DEWS; 2007) defined dry eye as a multifactorial disease of the tears and ocular surface system producing discomfort, visual disturbance, and tear film instability potentially leading to ocular surface system damage. In addition, dry eye disease is further characterized by increased osmolarity of the tear film and inflammation of the ocular surface.1 Dry eye also impacts daily activities such as reading, driving, work especially involving computer use, and social activities with significant effects on patient quality of life.2,3

Dry eye disease is a burden worldwide. Estimates of the global prevalence in individuals aged 50 years or older range from 5% to 30%.4 In Japan and China, dry eye has been reported to affect up to 22% of adults ages 40 years and older,5,6 and as many as 60% to 70% of young to middle-aged Japanese video terminal users.7 Among Asian patients older than 65 years, prevalence may be as high as 60% or 80%, depending on whether diagnosis is based on...
meibomian gland dysfunction or abnormal tear stability, respectively. Age, contact lens wear, ocular surgery, diet, smoking, history of allergy or diabetes, as well as environmental stimuli have been reported as risk factors leading to tear film instability and development of dry eye.

Chronic, T-cell–mediated inflammation involving the ocular surface and lacrimal glands has been demonstrated to have a prominent role in the development and progression of dry eye disease. Stress to the ocular surface activates signaling pathways in a variety of cell types, leading to expression of proinflammatory proteins (including cytokines/chemokines, proteinases, growth factors, and other mediators), further damage, and the development of a self-perpetuating inflammatory cycle. Punctal occlusion is a common treatment option for patients with dry eye, and improvements in clinical signs and symptoms have been reported after insertion of punctal plugs. However, there is limited evidence that punctal occlusion alters the underlying pathologic inflammation affecting tear film stability and cytokine composition in dry eye disease. Insertion of punctal plugs has been shown to temporarily reduce tear clearance, which may affect cytokine levels and inflammation. In patients with conjunctival chalasis or nasal conjunctivochalasia, a common ocular surface condition with delayed tear clearance, higher levels of interleukin (IL)-6, IL-8, IL-1β, and tumor necrosis factor (TNF)-α have been reported.

The present longitudinal study evaluated the effects of punctal occlusion in patients with dry eye disease. Changes in the severity and frequency of symptoms and clinical signs of dry eye after bilateral insertion of punctal plugs were assessed with a focus on effects of occlusion on tear cytokine and matrix metalloproteinase (MMP) levels.

II. MATERIALS AND METHODS

This prospective, interventional study was conducted between October 2012 and January 2013 at the Singapore Eye Research Institute (ClinicalTrials.gov identifier: NCT01684436). The study was performed in accordance to principles of the Declaration of Helsinki, the International Conference on Harmonisation, Good Clinical Practice guidelines, and all applicable laws and regulations. The study protocol and one amendment to the protocol were reviewed and approved by the SingHealth Centralised Institutional Review Board, and all patients provided written informed consent prior to starting study treatment.

A. Patients

Study eligibility was determined at the screening visit. Patients (21 years of age or older) were enrolled if they had moderate dry eye disease with persistent symptoms (light sensitivity, gritty or scratchy sensation, burning or stinging, blurred vision, vision that fluctuates with blinking, and vision that improves with artificial tears) and two or more symptoms that were grade 3 or greater (i.e., occurring often or all the time), corneal fluorescein staining greater than mild in severity (i.e., any staining beyond a periphery of 1 mm from the limbus), tear film breakup time (TFBUT) ≤10 seconds, and Schirmer I (without topical anesthetic) test score ≤10 mm.

Exclusion criteria included severe meibomian gland dysfunction (i.e., no more than two meibomian plugs per eyelid and no significant meibomian orifice irregularity), steroids or cyclosporine use within the last 3 months, insertion of punctal plugs within the last 3 months or presence of punctal plugs at the start of the study, and contact lens use within the last 7 days prior to start of study or during the course of the study, ocular conditions (other than dry eye) requiring the use of eye drops, and pterygium or other small corneal/conjunctival lesions or degeneration (e.g., Salzmann nodular degeneration). Also excluded were patients who had undergone laser in situ keratomileusis within the prior year; cataract or other ocular surgery within the last 3 months; corneal grafts; systemic disease such as diabetes, Sjögren syndrome, rheumatoid arthritis, or thyroid disease; and women of childbearing age who were nursing, pregnant, or considering becoming pregnant.

B. Punctal Plug Insertion and Clinical Assessments

Punctal plugs (Parasol® Punctal Occluder; Odyssey Medical, Beaver-Visitec International, Inc., Waltham, MA, USA) were inserted into the lower punctum of both eyes after baseline assessments. In the event that punctal plugs were lost during the course of the study, patients returned within 2 days for reinsertion of new plugs. In cases in which the loss of punctal plugs was not noticed, only data collected up to the last known presence of the plug was recorded and used for analysis. Patients were allowed to continue the use of lubricating tear drops, but cyclosporine, steroids, and/or doxycycline were not permitted during the course of the study.

At baseline prior to insertion of punctal plugs, and at week 1 and week 3 visits, patients had dry eye symptoms, Schirmer I test, TFBUT, and corneal fluorescein staining assessments, and underwent routine anterior segment slit-lamp microscope evaluation performed in the specific
sequence. Patients were requested not to administer lubricating tear drops within 30 minutes of the study visit. Dry eye symptoms were evaluated using a visual analog scale. Scores for frequency and severity of symptoms were collected via a patient questionnaire and recorded separately on a 100-mm line corresponding to the degree of the symptom, and global scores for irritation, blurred vision, and light sensitivity were calculated with higher scores indicating greater severity of disease.19

Prior to any anterior segment assessments, Schirmer I test was performed and the extent of wetting of each strip placed over the inferior temporal half of the lower lid margin in each eye was measured and recorded over 5 minutes. The Schirmer test strips were also used to collect tear protein samples (see Section II.C). TFBUT was measured after instillation of the fluorescein dye prior to assessing corneal staining. Fluorescein dye was instilled using floret strips (Laboratories Chauvin S.A., Aubenas, France) after wetting with a drop of normal saline and shaking off excess fluid. Corneal staining was then assessed in five corneal zones (central, superior, inferior, nasal, and temporal) following instillation of fluorescein dye, and graded on a scale of 0–4 (half-unit increments were allowed).20 The eye with the more severe disease was determined based on Schirmer test, TFBUT, or corneal fluorescein staining, and was designated the study eye.

Safety was assessed by monitoring adverse events, intraocular pressure, and visual acuity measurements, as well as questions on pain and slit-lamp examination of swelling around the punctal area.

C. Tear Inflammatory Protein Assessments

Levels of 15 cytokines and MMP-9 were analyzed in tear samples using a bead-based indirect immunofluorescent assay (Beadlyte®; EMD Millipore, Billerica, MA, USA) as described previously.21,22 Tear protein samples were collected at the same time and using the same test strips as the Schirmer test. At baseline and the week 1 and 3 study visits, tear proteins were collected prior to TFBUT and corneal staining to ensure that proteins were not washed out by reflex tearing. Schirmer test strips with tear protein samples from both eyes were cut in half (one half to be used for tear cytokine level analysis and one half for proteomics analysis) and stored in 1.5-mL Eppendorf tubes at −80°C until the time of analysis. Tear samples were diluted in 50-μL assay buffer and agitated for 20 minutes at 4°C, then 10-μL aliquots of the diluted samples were assayed. The multiplex bead-based assay detects free, total, and non-degraded forms of the cytokines and MMP-9.

D. Data Analysis and Statistical Methods

Study data were collected and managed by the Singapore Eye Research Institute trial management team. Changes in global symptom scores, corneal fluorescein staining, TFBUT, Schirmer test, and tear cytokines and MMP-9 levels were evaluated from baseline up to 3 weeks following insertion of punctal plugs. For cytokine and MMP-9 analyses, concentrations were normalized to the amount of wetting (plus 3 mm) of Schirmer test strips used to collect tears for the multiplex bead-based assays. Univariate analyses of individual parameters, as well as multivariate analyses adjusting for covariates,
were performed for clinical outcomes and cytokine levels. Changes in cytokine levels were also assessed in subgroups of patients demonstrating improvement in global irritation symptoms score versus those showing no improvements in global irritation symptoms score following punctal occlusion. Responders were defined as patients with improvement in symptoms after occlusion, where global symptom scores at week 3 minus week 0 were <0.

All statistical analyses were performed using SPSS for Windows, Version 12.0 (IBM Corporation, Armonk, NY, USA) and P<.05 was used to determine differences that were statistically significant. As this was a pilot study, no power analysis and sample-size calculations were performed.

III. RESULTS

A. Patients and Baseline Characteristics

A total of 30 patients were enrolled and had punctal plugs inserted bilaterally in the lower puncta. This report is based on 29 patients who completed the study; 1 patient withdrew consent following the baseline visit. The mean (standard deviation [SD]) age was 49.8 (14.1) years and the majority of patients were female (n=23). Female patients had a worse global symptoms irritation score (P=.03) than male patients at baseline. Corneal fluorescein staining assessed in all zones at baseline was not significantly different in female and male patients. In contrast, at baseline, males had worse TFBUT than females (P=.04). No other significant differences were observed in dry eye signs or symptoms between the groups at baseline (Table 1). Lubricating eye drop use prior to study enrollment is summarized in Table 2.

B. Effects of Punctal Occlusion on Clinical Symptoms and Signs of Dry Eye

At baseline, global irritation symptoms score (mean [SD]) was 53.8 (26.5) and decreased to 36.2 (24.9) after 3 weeks of punctal occlusion (Figure 1, top). The change in irritative symptoms from baseline was statistically significant. Blurred vision scores (middle) and light sensitivity scores (bottom) did not change significantly after punctal occlusion.

**Table 2.** Patients’ use of lubricating eye drops prior to enrollment

<table>
<thead>
<tr>
<th>Previous treatments</th>
<th>Number of patients</th>
<th>Daily use (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial tears (without preservatives)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear Naturale Free</td>
<td>10</td>
<td>&lt;1–5</td>
</tr>
<tr>
<td>Refresh Endura</td>
<td>3</td>
<td>1–12</td>
</tr>
<tr>
<td>Refresh Plus</td>
<td>3</td>
<td>1–4</td>
</tr>
<tr>
<td>Refresh Classic</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Artificial tears (with preservatives)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systane Ultra</td>
<td>4</td>
<td>1–20</td>
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<tr>
<td>Hialid</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Eyemo</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Hypomellose</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Eye ointment/gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duratears ointment</td>
<td>7</td>
<td>&lt;1–2</td>
</tr>
<tr>
<td>Vidisc gel</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Blephagel</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>No prior treatment</td>
<td>5</td>
<td>–</td>
</tr>
</tbody>
</table>

* Patients may have used more than one lubricating eye drop prior to baseline.
† <1 indicates occasional non-daily use of lubricating eye drops.

**Figure 1.** Mean global symptom scores for irritation, blurred vision, and light sensitivity following punctal occlusion. Global irritation symptoms scores (top) decreased from baseline up to 3 weeks after insertion of punctal plugs. Blurred vision scores (middle) and light sensitivity scores (bottom) did not change significantly after punctal occlusion. Data points represent mean ± standard error. *P<.001 compared with baseline by general linear model (repeat measures) analysis.
significant at the week 3 visit ($P<.001$) based on general linear model (repeat measure) analysis. Both males and females demonstrated improvements in global irritative symptoms over time, with the change in irritative symptoms remaining significant after adjusting for age and gender ($P=.02$). Compared with baseline, global blurred vision and light sensitivity symptom scores remained relatively the same after punctal occlusion (Figure 1, middle and bottom).

At 3 weeks, corneal staining was significantly decreased from baseline in the central, nasal, superior, and temporal corneal zones ($P<.01$), but not in the inferior zone ($P=.42$) (Figure 2). In addition, no statistically significant improvements were observed at week 1 or week 3 compared with baseline in either Schirmer I test scores or TFBUT (Figure 3, top and bottom).

C. Effects of Punctal Occlusion on Tear Cytokine and MMP-9 Levels

Cytokine and MMP-9 levels at baseline and after 1 week and 3 weeks of punctal occlusion are presented in Table 3. At week 3, cytokines and MMP-9 levels were similar to levels at baseline. Analysis of the relationship between baseline cytokine and MMP-9 levels and clinical signs and symptoms revealed that patients with low baseline Schirmer I test scores ($\leq 8$ mm) had elevated levels of tear cytokines and MMP-9 compared with patients with higher baseline Schirmer I test scores ($>8$ mm) (Table 4). Similarly, patients with baseline Schirmer I test scores $\leq 5$ mm had higher cytokine and MMP-9 levels compared with patients with baseline Schirmer I test scores $>5$ mm (data not shown). Cytokine levels that were higher in patients with low Schirmer I test scores at baseline remained elevated up to 3 weeks after punctal occlusion. No associations were found between cytokine or MMP-9 levels and global irritation symptoms scores or TFBUT at baseline.

The following associations were observed between baseline cytokine levels and corneal staining at baseline, but there were no consistent patterns in cytokine levels across the five corneal zones assessed: superior corneal staining was associated with IL-6 ($P=.026$) and TNF-$\alpha$ ($P=.001$) levels; inferior corneal staining was associated with IL-1$\beta$ ($P=.005$) and IL-2 ($P=.033$) levels; and temporal corneal

![Figure 2. General linear model (repeat measure) analysis of corneal staining. Corneal fluorescein staining significantly decreased after punctal occlusion in central, nasal, superior, and temporal zones ($P<.01$), but not in the inferior zone ($P=.42$), after 3 weeks of punctal occlusion. Data points represent mean $\pm$ standard error.]

![Figure 3. General linear model (repeat measures) analysis of Schirmer I test scores and tear film breakup time (TFBUT) changes following punctal occlusion. No statistically significant improvement in Schirmer I test scores (top) and TFBUT (bottom) were observed up to 3 weeks after punctal occlusion. Data points represent mean $\pm$ standard error.]

The following associations were observed between baseline cytokine levels and corneal staining at baseline, but there were no consistent patterns in cytokine levels across the five corneal zones assessed: superior corneal staining was associated with IL-6 ($P=.026$) and TNF-$\alpha$ ($P=.001$) levels; inferior corneal staining was associated with IL-1$\beta$ ($P=.005$) and IL-2 ($P=.033$) levels; and temporal corneal
staining was associated with IL-12 (P=0.018) and IL-13 (P=0.021) levels. These associations were not significant when adjusted for age.

Linear mixed-model analyses revealed that changes in interferon-gamma-inducible protein (IP)-10 (P<0.001) and macrophage inflammatory protein (MIP)-1α (P=0.032) levels after insertion of punctal plugs were associated with global irritation symptoms score. Higher levels of MIP-1α (but not IP-10) at baseline were observed among patients who demonstrated improvement in global irritation symptoms score following punctal occlusion compared with those patients without improvements in their global irritation symptoms score (Figure 4, top and bottom).

D. Safety

No adverse events were reported following bilateral insertion of punctal plugs during the course of the 3-week study.

IV. DISCUSSION

Inflammation of the ocular surface has been shown to play a critical role in the pathogenesis of dry eye. Changes in tear cytokine and MMP-9 levels have been reported in patients with dry eye disease or dysfunctional tear syndrome compared with asymptomatic healthy controls, including increased levels of IL-1β, TNF-α, interferon (IFN)−γ, IL-6, IL-8, IL-16, and MMP-9.23-26 In the earlier study by Lam et al, significant correlations were observed between IL-6 levels and ocular irritation symptoms, and between a number of cytokines and chemokines and clinical parameters including Schirmer I test scores, corneal fluorescein staining, and conjunctival lissamine green staining in patients with dysfunctional tear syndrome with or without meibomian gland disease. Treatment recommendations for dry eye disease developed by the International Task Force Delphi Panel emphasize that inflammation either triggers or maintains dry eye in most cases, even in the absence of clinically evident inflammation. A major focus of this study was the impact of punctal occlusion on ocular inflammation as reflected in tear cytokine levels.

Longitudinal changes in tear cytokine levels have been evaluated in daily contact lens wearers and in individuals using contact lens solutions, in patients administered preservative-free or preserved timolol 0.5% eye drops and subconjunctival anti-vascular endothelial cell growth factor injections, in patients with allergic keratoconjunctivitis secondary to nasal allergy, and following femtosecond laser-assisted laser in situ keratomileusis or small-incision lenticule extraction (SMILE).
lenticule extraction and corneal graft procedures. Punctal occlusion, a common treatment option for patients with dry eye, has been shown to improve clinical signs and symptoms. Changes in tear cytokine levels and associations with signs and symptoms after insertion of punctal plugs have not been fully elucidated.

After bilateral punctal plug insertion in our study, patients with dry eye demonstrated improvements in irritative symptoms, as well as reductions in central, superior, nasal, and temporal corneal staining. Increased tear retention following punctal occlusion may have improved wound healing due to reduced frictional forces during blinking, which could have contributed to the reduction in corneal epithelial staining. However, there was no change in inferior zone corneal staining. The inferior cornea where initial tear breakup occurs is prone to tear film instability due to meniscus suction effects and as a result, may be more resistant to improvements in corneal staining. Despite some of these improvements in symptoms and signs, the overall tear cytokine levels were not significantly altered following insertion of punctal plugs.

A low Schirmer I test score (<8 mm) was found to be the factor most consistently associated with higher cytokine levels at baseline. Cytokine levels elevated at baseline in patients with low Schirmer I test scores remained high after punctal occlusion, including the T helper type 1 cytokines TNF-α, IL-1β, and IFN-γ. There was no association between baseline cytokine or MMP-9 levels and baseline global irritation symptoms scores or TFBUT, and no consistent associations with baseline fluorescein staining. Multivariate analyses revealed some association between changes in global irritative symptoms with changes in IP-10 and MIP-1α cytokine levels after punctal occlusion. Adverse events typically experienced with punctal plugs, such as epiphora, foreign body sensation, eye irritation, and spontaneous plug loss, were not reported during this 3-week study.

Control cases with patients using eye drops only with no punctal occlusion were not included, since our study was not evaluating the therapeutic efficacy of punctal plugs compared to lubricating drops which has been addressed in other studies. Limitations of this study include its small sample size and short duration. Additional studies enrolling a larger population conducted over a longer period of time are needed to confirm the effects of punctal occlusion that were observed on tear protein levels and
inflammation, and the association with other disease characteristics. Studies evaluating concurrent treatment with punctal plugs and anti-inflammatory eye drops are also warranted. The use of Schirmer test strips to collect tear protein samples may also have inadvertently included proteins from the conjunctival epithelium, which may have resulted from injury during test strip-insertion. Tear meniscus height and conjunctivochalasis, which were not measured in this study, could be assessed in future studies evaluating effects of punctal occlusion on tear inflammatory proteins.

Our findings indicate that ocular inflammation is not relieved by insertion of punctal plugs. Blockage of tear drainage using punctal plugs may have resulted in retention of proinflammatory proteins, leading to pooling of cytokines in the lower fornix and subsequent damage to the ocular surface from ongoing inflammation. This mechanism may be responsible for the persistent corneal staining in the inferior zone seen in patients evaluated in our study. Similarly, in a 17-day study of the effects of punctal occlusion, decreased tear clearance was observed in the first 3 days after insertion of punctal plugs. However, improvement in tear clearance observed from day 3 to the study end suggests that a reduction in tear clearance is unlikely to be the main cause of the continued high levels of cytokines observed up to 3 weeks after occlusion in our study. There is also a possibility that mechanically induced inflammation, due to contact between the punctal occluder and the canalicul wall, triggered production of cytokines and this may negate any improvements in inflammation from the retention of tears.

V. CONCLUSIONS

Punctal plugs do not directly address the underlying ocular surface inflammation in dry eye disease. Novel treatment strategies with cyclosporine, topical corticosteroids, tacrolimus, tetracycline derivatives, and other anti-inflammatories have been developed to target underlying inflammatory pathways in dry eye. The lack of significant changes in cytokine levels and inferior corneal staining following punctal occlusion observed in this study suggests that patients with dry eye may benefit from earlier treatment or concurrent instillation of anti-inflammatory agents with punctal plugs for management of their disease.

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