Dry Eye: an Inflammatory Ocular Disease

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Keratoconjunctivitis sicca, or dry eye, is a common ocular disease prompting millions of individuals to seek ophthalmological care. Regardless of the underlying etiology, dry eye has been shown to be associated with abnormalities in the pre-corneal tear film and subsequent inflammatory changes in the entire ocular surface including the adnexa, conjunctiva and cornea. Since the recognition of the role of inflammation in dry eye, a number of novel treatments have been investigated designed to inhibit various inflammatory pathways. Current medications that are used, including cyclosporine A, corticosteroids, tacrolimus, tetracycline derivatives and autologous serum, have been effective for management of dry eye and lead to measurable clinical improvement.

Keywords: Keratoconjunctivitis Sicca; Sjögren’s Syndrome; Dry Eye; Inflammation; Treatment

OVERVIEW

Although often disregarded as a minor problem, keratoconjunctivitis sicca, commonly referred to as dry eye, is a growing public health concern affecting as many as 17% of women and 11.1% of men in the United States.1 This is likely an underestimate if one also considers self-treating patients and milder/periodic cases with intermittent symptomaticology.

A recent international Dry Eye Workshop (DEWS) defined dry eye as a “multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface which is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.”2 Identification of inflammation as a major factor in dry eye helped make a tremendous step forward in the description and treatment of this condition.

The DEWS also recognized two subgroups of dry eye based on etiopathogenesis: aqueous deficient and evaporative. Among the aqueous deficient group, there are two major subclasses: Sjögren’s syndrome (SS) dry eye and non-SS dry eye. Diagnosis of SS is generally made based on the American–European Consensus Group 2002 revised classification criteria, requiring at least four out of six criteria, or three out of the four objective criteria, to be present. The six criteria include: subjective and objective ocular dryness; subjective and objective oral dryness; presence of Sjögren-specific antibody A (SSA)/Ro and/or Sjögren-specific antibody B (SSB)/La; and positive minor salivary gland biopsy.3 However, in 2012, a new classification criteria for SS was endorsed by The American College of Rheumatology that requires at least 2 of the following 3 criteria: 1) positive serum anti-SSA and/or anti-SSB or rheumatoid factor or antinuclear antibody (titer >1:320), 2) total ocular surface staining score >3, and 3) presence...
of focal lymphocytic sialadenitis with a focus score >1/4 mm² in labial salivary gland biopsy samples.⁴

According to the classification criteria from the European-American collaboration, secondary SS (sSS) consists of features of primary SS (pSS) together with features of an overt autoimmune connective tissue disease, the most common of which is rheumatoid arthritis. There is a well-known association of several systemic diseases with dry eye syndrome such as SS, rheumatoid arthritis, scleroderma, polymyositis, lymphoma, amyloidosis, hemochromatosis, sarcoidosis, and systemic lupus erythematosus.⁵ Although the rate of dry eye in various inflammatory diseases is known, the frequency of associated systemic rheumatic conditions in patients with dry eye is currently unknown. A previous retrospective study from a single tertiary eye care center determined that pSS is underdiagnosed and should be the focus of diagnostic evaluations in individuals with clinically significant aqueous deficient dry eye. Only 33.3% of patients with pSS carried the diagnosis at the time of presentation and 50% were diagnosed as a result of the initial evaluation.⁶ A more recent multicenter prospective study confirmed these findings in a group of more than 300 patients with clinically significant dry eye and found the rate of SS to be 11.6%.⁷ The difference in the rate of SS between these two studies could perhaps be attributed to the fact that the prospective study was limited in regards to the diagnostic tests performed: minor salivary gland biopsy or tests for objective dry mouth findings were not utilized. Nonetheless, both studies concluded that ophthalmologists managing patients with clinically significant dry eye should have a high index of suspicion for underlying SS and a low threshold for diagnostic work-up.

Previously unrecognized autoimmune thyroid disease has also been shown to be a cause of inflammatory ocular surface disease with dry eye symptomatology and should be considered when evaluating patients with dry eye. A retrospective, observational case series of 539 patients referred for dry eye evaluation has confirmed this correlation; of the 32 patients who underwent standardized orbital echography with a clinical suspicion, 21 (66%) were diagnosed with occult thyroid eye disease.⁸

On the other hand, based on multiple epidemiological studies, older age and female sex are widely recognized as the two most common risk factors for dry eye.⁹,¹⁰ Peri- and postmenopausal females seem to be particularly at a higher risk. This perhaps suggests that dry eye is an involutional disorder. In addition, hormonal studies demonstrate that sex hormones influence ocular surface conditions through their effects on aqueous tear secretion, meibomian gland function, and conjunctival goblet cell density.¹¹,¹² Thus, an altered hormonal state (e.g., following menopause) may be blamed to cause dry eye. Several other external factors are also known to precipitate and exacerbate dry eye, such as long-term contact lens wear, refractive laser surgery, smoking, and extended visual tasks like computer use, watching television and prolonged reading.¹³–¹⁵ Worsening of dry eye may also be attributed to low relative humidity conditions that are common in office environments, air-conditioned cars, airplane cabins, and extreme hot or cold weather.¹⁶ Dry eye may be caused by systemic medications with anticholinergic effects (e.g. antihistamines, antidepressants, antipsychotics) as well as diuretics.¹⁷ Frequent instillation (>4–6 times daily) of preserved eye drops, particularly with benzalkonium chloride for example for glaucoma, may also contribute to dry eye because of their well-established ocular surface toxicity.¹⁷

Irrespective of the presence of any identifiable underlying local or systemic inflammatory disorder, dry eye seems to be invariably associated with chronic inflammation of the ocular surface, as detailed below, although it is not known whether the local inflammation is causative or simply occurs as a consequence of ocular dryness. Nevertheless, recognition of the role of inflammation in dry eye has been a crucial factor in facilitating dry eye treatment.

**PATHOPHYSIOLOGY**

There is growing evidence from the past decade indicating that dry eye-related ocular surface inflammation is mediated by lymphocytes.¹⁸ Based
on earlier immuno-histopathological evaluations, patients with both SS-related as well as non-SS dry eye have identical conjunctival inflammation manifested by T cell infiltrates and upregulation of CD3, CD4, and CD8 as well as lymphocyte activation markers CD11a and HLA-DR. These results suggested that clinical symptoms of dry eye may be dependent on T-cell activation and resultant autoimmune inflammation. Multiple other studies followed and demonstrated the role of pro-inflammatory cytokines and matrix metalloproteinases (MMPs) in the pathogenesis of dry eye. Interleukin (IL)-1 is one of the most widely studied cytokines accompanying dry eye. An increase in the pro-inflammatory forms of IL-1 (IL-1α and mature IL-1β) and a decrease in the biologically inactive precursor IL-1β have been found in the tear film of dry eye patients. The source of increased levels of IL-1 was thought to be the conjunctival epithelium based on immunohistochemical studies. More recently, reactive nitrogen species expressed by conjunctival epithelium have been recognized in the pathogenesis or self-propagation of SS-related dry eye. In the same study, IL-1β, IL-6, IL-8 and tumor necrosis factor (TNF) α were also investigated and found to play a significant role in SS-related dry eye as compared to normal eyes. The response of cells to extracellular stimuli such as ocular surface stress, including changes in the composition of tear film or hyperosmolarity and ultraviolet light exposure, is mediated in part by a number of intracellular kinase and phosphatase enzymes. Mitogen-activated protein (MAP) kinases are integral components of parallel MAP kinase cascades activated in response to a number of cellular stresses including inflammatory cytokines (e.g., IL-1 and TNF-alpha), heat shock protein, bacterial endotoxin and ischemia. Activation of these MAP kinase homologues mediates the transduction of extracellular signals to the nucleus and is pivotal in regulation of the transcription events that determine functional outcomes in response to such stresses. These stress-activated protein kinases have been identified in the tear film of patients with dry eye. It has been documented that activation of these stress pathways results in transcription of stress-related genes, including MMPs, mainly MMP-9. In another study, MAP kinases were found to stimulate the production of inflammatory cytokines including IL-β, TNF-α, and MMP-9 and thereby cause ocular surface damage. As previously mentioned, hyperosmolarity is one of the factors contributing to ocular surface inflammation. Hyperosmolarity induces inflammation in human limbal epithelial cells by increasing expression and production of pro-inflammatory cytokines and chemokines such as IL-1β, TNF-α, and the C-X-C chemokine IL-8. This process appears to be mediated through activation of the c-Jun N-terminal kinases and MAPK signaling pathways. All of these inflammatory mediators and pathways should not only be considered important as they relate to the pathogenesis of dry eye; they should also be kept in mind when discussing treatment strategies.

**TREATMENT**

As it is widely recognized that inflammation has a significant role in the etiopathogenesis of dry eye, promoting ocular surface disruption and symptoms of irritation, a number of anti-inflammatory treatments are currently in use for its management. Many more anti-inflammatory medications are in development or clinical trial phases. These agents inhibit the expression of inflammatory mediators on the ocular surface, thereby restoring the secretion of a healthy tear film and reducing signs and symptoms.

**Cyclosporine A**

The immunomodulating effects of cyclosporine A are achieved through binding with cyclophilins, which are a group of proteins. Cyclophilin A which is found in the cytosol, and the cyclosporine-cyclophilin A complex inhibits a calcium/calmodulin-dependent phosphatase, calcineurin, the inhibition of which is thought to halt the production of the transcription of T-cell activation by inhibiting IL-2. Cyclophilin D is located in the matrix of mitochondria. Cyclosporine A-cyclophilin D complex modulates the mitochondrial permeability.
transition pore thereby inducing mitochondrial dysfunction and cell death.\textsuperscript{27} The reduction in inflammation, via inhibition of T-cell activation and down-regulation of inflammatory cytokines in the conjunctiva and lacrimal gland,\textsuperscript{28,29} is thus thought to enhance tear production.\textsuperscript{30–32} Topical cyclosporine also increases goblet cell density and decreases epithelial cell apoptosis.\textsuperscript{33} Commercially available topical cyclosporine 0.05\% (Restasis, Allergan, Irvine, CA, USA) or 1\% compounded preparations are frequently utilized for treatment of various inflammatory ocular surface disorders.\textsuperscript{34} Dosing topical cyclosporine at a frequency greater than twice a day may be more effective for patients who do not demonstrate improvement of severe dry eye disease with the twice-daily regimen.\textsuperscript{8,35}

**Tacrolimus**

This topical anti-inflammatory agent (previously known as FK506) is a macrolide antibiotic isolated from *Streptomyces tsukubaensis* fermentation.\textsuperscript{36} Although the mechanism of action of tacrolimus is similar to cyclosporine A, its potency in vitro has been shown to be significantly greater, exhibiting similar effects at 100 times lower concentrations.\textsuperscript{37} Only when bound to immunophilin does it become biologically active, thus effectively inhibiting calcineurin, and inhibiting T and B lymphocyte activation via reduction in IL-2 synthesis.\textsuperscript{38–44} Tacrolimus suppresses the immune response by inhibiting the release of other inflammatory cytokines as well (e.g., IL-3, IL-4, IL-5, IL-8, interferon-gamma, and TNF-alpha).\textsuperscript{45–48} Systemic tacrolimus has been reported to be effective for improving dry eye associated with graft versus host disease; however, there are potential adverse reactions to be aware of when administering long-term systemic therapy.\textsuperscript{49} Topical tacrolimus, available as 0.03\% and 0.1\% ointments as well as compounded eye drops, is promising for the treatment of dry eye in the setting of chronic graft versus host disease and SS.\textsuperscript{50–52}

**Corticosteroids**

Topical steroids, through several mechanisms of action, help reduce ocular inflammation. Corticosteroids function via suppression of cellular infiltration, capillary dilation, proliferation of fibroblasts, and collagen deposition. They stabilize intracellular and extracellular membranes. Corticosteroids increase the synthesis of lipocortins that block phospholipase A\textsubscript{2} and inhibit histamine synthesis in mast cells.\textsuperscript{53} Inhibition of phospholipase A\textsubscript{2}, an essential step in the inflammatory cascade, prevents the conversion of phospholipids to arachidonic acid. Corticosteroids also interfere with transcription factor NF-kB, which regulates the synthesis of a number of pro-inflammatory molecules, thereby stimulating lymphocyte apoptosis. Corticosteroids mediate their anti-inflammatory effects primarily through modulation of the cytosolic glucocorticoid receptor at the genomic level.\textsuperscript{54,55} After corticosteroids bind to the glucocorticoid receptor in the cytoplasm, the activated corticosteroid-glucocorticoid receptor complex migrates to the nucleus, where it up-regulates the expression of anti-inflammatory proteins and represses the expression of pro-inflammatory proteins. However, recent work suggests that the activated corticosteroid-glucocorticoid receptor complex also elicits non-genomic effects, such as inhibition of vasodilation, vascular permeability and migration of leukocytes.\textsuperscript{54,56}

Several clinical studies have demonstrated the effectiveness of topical steroids for treatment of dry eye. In a retrospective clinical series, topical administration of a 1\% solution of non-preserved methylprednisolone, given three or four times daily for several weeks to patients with SS related dry eye, provided moderate to complete relief of symptoms in all patients.\textsuperscript{57} In addition, there was a decrease in corneal fluorescein staining score (2.6±0.5 on a 12-point scale) and complete resolution of filamentary keratitis. This therapy was effective even for patients suffering from severe dry eye who had no improvement from maximum aqueous tear enhancement/replacement therapies. A pilot study on 64 patients was conducted evaluating the efficacy of loteprednol etabonate (LE) 0.5\% ophthalmic suspension 4 times a day versus placebo for treatment of the inflammatory component of dry eye associated with aqueous tear deficiency and delayed tear clearance.\textsuperscript{58}

\textsuperscript{27} Hessen and Akpek, J O U R N A L O F  O P H T H A L M I C A N D  V I S I O N  R E S E A R C H  2014; Vol. 9, No. 2
After 2 weeks of therapy in the subset of patients with moderate to severe clinical inflammation, a significant difference was observed between LE-treated group and vehicle-treated group in central corneal staining, nasal bulbar conjunctival hyperemia, and lid margin injection. None of the patients experienced a clinically significant increase in intraocular pressure following one month of therapy. Patients treated with topical corticosteroids should be monitored closely for known risks of cataract formation, glaucoma, corneal thinning and infectious keratitis.59

Tetracycline Derivatives

Tetracycline derivatives uniquely possess antibacterial as well as anti-inflammatory properties. Doxycycline has been shown to inhibit c-Jun N-terminal kinase and extracellular signal-related kinase mitogen-activated protein kinase signaling in epithelial cells of the ocular surface exposed to hyperosmolar stress, down-regulating the expression of CXCL8 and pro-inflammatory cytokines IL-1β and TNF.60 Doxycycline inhibits MMP-9 activity and supports ocular surface integrity.61,62 Additionally studies demonstrated that minocycline inhibits the expression of cell-associated pro-inflammatory molecules, including major histocompatibility complex class II.63 Doxycycline has been reported to be effective in patients with ocular rosacea by reducing irritation symptoms, improving tear film stability, and decreasing the severity of ocular surface disease.64–66 In addition, doxycycline has been useful in the treatment of corneal erosions.67,68

Autologous Serum

Serum contains several anti-inflammatory factors that have the capability to inhibit soluble mediators of the ocular surface inflammatory cascade associated with dry eye. These include inhibitors of inflammatory cytokines (e.g., IL-1 RA and soluble TNF-receptors) and MMP inhibitors (e.g., TIMPs).69–71 Clinical trials have shown that autologous serum drops improve ocular irritation symptoms, and conjunctival and corneal dye staining in dry eye that occurs in the setting of SS.72–74 Conversely, there is greater risk of microbial growth as autologous serum drops, in addition to antimicrobial agents, contain high protein content and are generally non-preserved.75

Recent studies have investigated cord serum drops (prepared from donor umbilical cord serum) as well as allogenic serum drops (from a related donor). A clinical trial included 17 patients with GVHD- and 13 patients with SS-associated dry eye treated for 1 month with cord blood serum. Patients received cord blood once a day (containing 0.15 ng epithelial growth factor per drop). Patients reported a decrease in discomfort symptoms as measured with the Ocular Surface Disease Index score (OSDI) (22.3±10.3 vs. 39.3±16.9). Also clinical findings such as impression cytology score (3.8±1.2 vs. 6.6±2.1), tear osmolarity (312.5±7 vs. 322±9.1 mOsm/L), and corneal sensation (measured with Cochet-Bonnet esthesiometer) (48.2±2.1 vs. 49.7±2.1 nylon/mm/length) improved significantly.76 Another study involving 12 patients with chronic GVHD-associated severe dry eye treated with cord blood serum for a period of 6 months reported statistically significant improvement (P<0.01) in symptom score (on a scale of 0-4, from 3.83±0.38 to 0.83±0.57), corneal sensitivity (from 52.08±6.06 mm to 57.50±3.00 mm), tear breakup time (BUT) (from 2.50±0.91 to 5.71±1.04 seconds), and corneal fluorescein staining (from 7.42±2.02 to 1.29±0.46).77 Also shown to be effective are allogenic serum drops, prepared using blood from a family member rather than the patient’s own blood. Allogeneic serum tears were used for the treatment of dry eye in patients with GVHD. After 4 weeks of continuous use, significant improvement was noted in symptom scores (as measured by OSDI Score from 32.5 to 8.9), tear osmolarity (from 311.1 to 285.1 m osmol), corneal staining (from 2.5 to 1.8) as well as increased goblet cell density (from 90.6 to 122.6 cell/mm²) and tear BUT (from 2.9 to 4.4 seconds).78

IL-Ra

Interleukin-1 receptor antagonist (IL-1Ra) is an endogenous IL-1 receptor blocker primarily
produced by activated monocytes and tissue macrophages which inhibits the activities of the pro-inflammatory forms of IL-1 (IL-1α and IL-1β) by competitively binding to the IL-1 receptor-I. In a murine model with environmentally induced dry eye, a significant decrease in corneal fluorescein staining was observed with slit lamp biomicroscopy after topical treatment with 3 microliters of IL-Ra applied 3 times daily for 9 days. Comparison treatments, 1% methylprednisolone and 0.05% cyclosporine A, were equally effective in this model. Additionally, confocal microscopy revealed a significant decrease in the number of central corneal CD11b+ cells, lymphatic growth and interleukin-1β expression after treatment with 5% IL-1Ra and 1% methylprednisolone, but not with cyclosporine A. This suggests that IL-1Ra is comparable to topical methylprednisolone in reducing inflammation and improving clinical signs of dry eye.

**Resolvin E1 (Rx-10001)**

Resolvin E1 (RvE1) is a new class of endogenous immune response mediators derived from the lipoxygenation of the essential dietary omega-3 polyunsaturated fatty acids, eicosapentaenoic acid, and docosahexaenoic acid. In animal models, treatment applied 4 times per day for one week, using topical 100μg/mL (0.01%) omega-3 derivatives has been shown to reverse corneal epithelial damage associated with dry eye. A specialized corneal tomography module (Rostock Cornea Module of the Heidelberg Retina Tomograph) was used to study the corneas in vivo. Increased tear flow promoting a healthy epithelium, decreased cyclooxygenase-2 expression by Western Blot Analysis, and decreased macrophage infiltration were also noted. In a murine model of dry eye it was shown that RvE1, delivered topically at 300μg/ml concentration 4 times a day, improved corneal staining and goblet cell density. The synthetic analog of RvE1 (RX-10045) is being tested in a Phase II clinical trial for treatment of chronic dry eye. Preliminary data of a 28-day, randomized, placebo-controlled, 232-patient trial showed dose-dependent and statistically significant improvement using RX-10045; however, final data have not been published.

**Chemokine Receptor Antagonist**

Monocyte chemotactic protein 1 is secreted by monocytes, memory T cells, macrophages, fibroblasts, endothelial cells and mast cells. It stimulates the movement of leukocytes along a chemotactic gradient after binding to its cell surface receptor chemokine receptor antagonist. The critical role of the coupled monocyte chemotactic protein 1/chemokine receptor antagonist in inflammation has been demonstrated using monocyte chemotactic protein 1 and chemokine receptor antagonist knockout mice, suggesting that inhibition of migration of chemokine receptor antagonist-bearing mononuclear cells may be an effective mechanism to modulate disease progression in chronic inflammation. A study of dry eye disease in a murine model, which received topical chemokine receptor antagonist (5.0 mg/ml) twice daily for 7 days, showed a significant decrease in corneal fluorescein staining. Real-time polymerase chain reaction revealed decreased infiltration of corneal CD11b(+) cells and conjunctival T cells compared with vehicle treated and untreated dry eye groups. The chemokine receptor antagonist also significantly decreased messenger RNA expression levels of IL1-alpha and 1-beta in the cornea, and TNF-alpha and IL1-beta in the conjunctiva.

**Tofacitinib (CP-690,550)**

Tofacitinib (CP-690,550) is a selective inhibitor of the janus kinase (JAK). Janus kinase signaling is essential for immune cell activation, pro-inflammatory cytokine production and cytokine signaling. Tofacitinib inhibits JAK1, JAK2, and JAK3 in vitro with functional cellular selectivity for JAK1 and JAK3 over JAK2. Inhibition of JAK1 and JAK3 by tofacitinib blocks signaling through the common γ chain containing receptors for several cytokines, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. In addition, inhibition of JAK1 will result in attenuation of signaling by additional pro-inflammatory cytokines, such as
IL-6 and interferon-γ. Tofacitinib subsequently modulates adaptive and innate immunity with limited effect on hematopoiesis. In Phase I/II trials, topical tofacitinib (CP-690,550) at a concentration of 0.0003%-0.005% was used in 327 patients with clinically significant aqueous deficient dry eye for a period of 8 weeks. A trend for improving both signs (Schirmer’s test without anesthesia and corneal fluorescein staining) and symptoms of dry eye, with a reasonable safety profile was noted. In addition, a sub-study of Phase I/II trials showed a reduction in inflammation assessed by change from baseline in conjunctival cell surface expression of human leukocyte antigen DR-1 studied by flow cytometry and tear levels of several cytokines and inflammation markers by microsphere-based immunoassays.

SAR 1118 (LFA-1 antagonist)

SAR 1118, a novel investigational small-molecule lymphocyte function-associated antigen-1 antagonist, was engineered for topical ophthalmic delivery. The binding of lymphocyte function-associated antigen-1 on the surface of T cells to intercellular adhesion molecule-1 on endothelial, epithelial, and antigen presenting cells is a critical step in T-cell activation (normal immune response and inflammation). Thus, it has been proposed that blockade of lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction may give a therapeutic benefit in patients with dry eye, breaking the chronic cycle of T-cell mediated inflammation and thus aiding in the recovery of the ocular surface. SAR 1118 is an effective inhibitor of T-cell activation, adhesion, migration, proliferation and cytokine release. A multicenter, prospective, double-masked, placebo-controlled trial included 230 dry-eye subjects randomized to receive SAR 1118 (0.1, 1.0, 5.0%) or placebo eye drops twice daily for 84 days. SAR 1118 showed dose-dependent and statistically significant improvement in corneal staining scores, symptoms measured with OSDI (both total ocular surface disease index and visual related function questions) as compared to placebo. Improvements in tear production and symptoms were noted as early as day 14. It was well tolerated and no serious ocular adverse events were reported. Several Phase III trials are underway and results are yet to be published.

Mapracorat

Mapracorat (formerly ZK-245186 and subsequently BOL-303242-X) is a novel selective glucocorticoid receptor agonist currently under investigation for its anti-inflammatory effects as it pertains to dry eye. The anti-inflammatory effects of mapracorat were assessed in an in vitro osmotic stress model which simulates some of the pathophysiological changes seen in dry eye. Incubation of cells with mapracorat 0.1-1.0% applied 3 times a day for 7-8 days inhibited hyperosmolar-induced cytokine release with comparable activity and potency as a commonly used steroid, dexamethasone. In addition, another study observed mapracorat to be effective in maintaining tear volume and tear break-up time with no increase in intraocular pressure in a rabbit model.

SUMMARY

Regardless of whether or not an underlying systemic inflammatory condition can be identified, dry eye seems to be associated with chronic and sometimes subclinical inflammation that might eventually cause ocular surface damage. Novel treatments targeting specific mediators in inflammatory reactions known to be associated with dry eye are currently evolving.

Conflicts of Interest

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REFERENCES

1. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol 2000;118:1264-1268.
2. The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international dry eye workshop (2007). Ocul Surf 2007;5:75-92.

3. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren syndrome: a revised version of the European criteria proposed by the American-European consensus group. Ann Rheum Dis 2002;61:554-558.

4. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology Classification Criteria for Sjögren’s Syndrome: A Data-Driven, Expert Consensus Approach in the SICCA Cohort. Arthritis Care Res (Hoboken) 2012;64:475-487.

5. Djililian AR, Hamrah P, Pflugfelder SC. Dry eye. In: Krachmer JH, Mannis MJ, Holland EJ (eds). Cornea. 2nd ed. Philadelphia. Elsevier Mosby; 2005: 521–540.

6. Akpek EK, Klimava A, Thorne JE, Martin D, Lekhanont K, Ostrovsky A. Evaluation of patients with dry eye for the presence of underlying Sjögren syndrome. Cornea 2009;28:493-497.

7. Liew M, Zhang M, Kim E, Akpek EK. Prevalence and predictors of Sjögren’s syndrome in a prospective cohort of patients with aqueous deficient dry eye. Br J Ophthalmol 2012;96:1498-1503.

8. Gupta A, Sadeghi PB, Akpek EK. Occult thyroid eye disease in patients presenting with dry eye symptoms. Am J Ophthalmol 2009;147:919-923.

9. The epidemiology of dry eye disease: Report of the Epidemiology Subcommittee of the International Dry Eye Workshop 2007;5:75-92.

10. Conner CG, Flockencier LL, Hall CW. The influence of gender on the ocular surface. J Am Optom Assoc 1999;70:182–186.

11. Krenzer KL, Dana MR, Ullman MD, Cermak JM, Tolls DB, Evans JE, et al. Effect of androgen deficiency on the human meibomian gland and ocular surface. J Clin Endocrinol Metab 2000;85:4874-4882.

12. Schaumberg DA, Buring JE, Sullivan DA, Dana MR. Hormone replacement therapy and dry eye syndrome. JAMA 2001;286:2114-2119.

13. Ang RT, Dartt DA, Tsubota K. Dry eye after refractive surgery. Curr Opin Ophthalmol 2001;12:318-322.

14. Lee AJ, Lee J, Saw SM, Gazzard G, Koh D, Widjaja D, et al. Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. Br J Ophthalmol 2002;86:1347–1351.

15. Schlote T, Kaden G, Frudenthaler N. Marked reduction and distinct pattern of eye blinking in patients with moderately dry eyes during video display terminal use. Graefes Arch Clin Exp Ophthalmol 2004;242:306–312.

16. Wolkoff P, Nøjaard JK, Franck C, Skov P. The modern office environments desiccate the eye? Indoor Air 2006;16:258–265.

17. Management and therapy of dry eye disease: Report of the Management and Therapy Subcommittee of the International Dry Eye Workshop (2007). Ocul Surf 2007;5:163–178.

18. Kunert KS, Tisdale AS, Stern ME, Smith JA, Gipson IK. Analysis of topical cyclosporine treatment of patients with dry eye syndrome: effect on conjunctival lymphocytes. Arch Ophthalmol 2000;118:1489–1496.

19. Stern ME, Gao J, Schwab TA, Ngo M, Tieu DD, Chan CC, et al. Conjunctival T-cell subpopulations in Sjögren’s and non-Sjögren’s patients with dry eye. Invest Ophthalmol Vis Sci 2002;43:2609–2614.

20. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 2001;42:2283–2292.

21. Cejková J, Ardan T, Simonová Z, Cejka C, Malec J, Jirsová K, et al. Nitric oxide synthase induction and cytotoxic nitrogen-related oxidant formation in conjunctival epithelium of dry eye (Sjögren’s syndrome) Nitric Oxide 2007;17:10–17.

22. Paul A, Wilson S, Belham CM, Robinson CJ, Scott PH, Gould GW, et al. Stress-activated protein kinases: activation, regulation and function. Cell Signal 1997;9:403–410.

23. Pflugfelder SC, de Paiva CS, Tong L, Luo L, Stern ME, Li DQ. Stress-activated protein kinase signaling pathways in dry eye and ocular surface disease. Ocul Surf 2005;3(Suppl 4):154–157.

24. Luo L, Li DQ, Doshi A, Farley W, Corrales RM, Pflugfelder SC. Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. Invest Ophthalmol Vis Sci 2004;45:4293–4301.

25. Li DQ, Luo L, Chen Z, Kim HS, Song XJ, Pflugfelder SC. JNK and ERK MAP kinases mediate induction of IL-1beta, TNF-alpha and IL-8 following hyperosmolar stress in human limbal epithelial cells. Exp Eye Res 2006;82:588–596.

26. Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. Immunopharmacology 2000;47:119–125.

27. Stevenson W, Chauhan SK, Dana R. Dry Eye Disease: an immune-mediated ocular surface disorder. Arch Ophthalmology 2012;130:90-100.
28. Pflugfelder SC, Wilhelmus KR, Osato MS, Matoba AY, Font RL. The auto-immune nature of aqueous tear deficiency. *Ophthalmology* 1986;93:1513-1517.

29. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal gland functional unit in the pathophysiology of dry eye. *Exp Eye Res* 2004;78:409-416.

30. Stevenson D, Tauber J, Reis BL. Efficacy and safety of cyclosporine A ophthalmic emulsion in the treatment of moderate to severe dry eye disease: a dose-ranging, randomized trial. The Cyclospoine A Phase 2 Study Group. *Ophthalmology* 2000;107:967-974.

31. Sall K, Stevenson OD, Mundorf TK, Reis BL. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. *Ophthalmology* 2003;107:631-639.

32. Laibovitz RA, Solch S, Andriano K, O'Connell L. Topical noncorticosteroid immunomodulation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. *Arch Ophthalmol* 2002;120:330-337.

33. Kunert KS, Tisdale AS, Gipson IK. Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. *Cornea* 1993;12:315-323.

34. Utine CA, Stern M, Akpek EK. Clinical review: topical ophthalmic use of cyclosporin A. *Ocul Immunol Inflamm* 2010;18:352-361.

35. Dastjerdi MH, Hamrah P, Dana R. High-frequency topical cyclosporine 1% ophthalmic ointment in the treatment of keratoconjunctivitis sicca. *Cornea* 1999;13:315-323.

36. Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther Drug Monit* 1995;17:584-591.

37. Kino T, Hatanaka H, Hashimoto M, Nishiyama M, Goto T, Okuhara M, et al. FK-506, a novel immunosuppressant isolated from Streptomyces. I. Fermentation isolation, and physio-chemical and biological characteristics. *J Antibiot (Tokyo)* 1987;40:1249-1255.

38. Attas-Fox L, Barkana Y, Ishakov V, Rayvich S, Gerber Y, Morad Y, et al. Topical tacrolimus 0.03% ointment for intractable allergic conjunctivitis: an open-label pilot study. *Curr Eye Res* 2008;33:545-549.

39. Bertelmann E, Pleyer U. Immunomodulatory therapy in ophthalmology—is there a place for topical application? *Ophthalmologica* 2004;218:359-367.

40. Fei WL, Chen JQ, Yuan J, Quan DP, Zhou SY. Preliminary study of the effect of FK506 nanospheric-suspension eye drops on rejection of penetrating keratoplasty. *J Ocul Pharmacol Ther* 2008;24:235-244.

41. Fujita E, Teramura Y, Mitsuji K, Ninomiya S, Iwatsubo T, Kawamura A, et al. Absorption, distribution, and excretion of 14C-labeled tacrolimus (FK506) after a single or repeated ocular instillation in rabbits. *J Ocul Pharmacol Ther* 2008;24:333-343.

42. Fujita E, Teramura Y, Shiraga T, Yoshioka S, Iwatsubo T, Kawamura A, et al. Pharmacokinetics and tissue distribution of tacrolimus (FK506) after a single or repeated ocular instillation in rabbits. *J Ocul Pharmacol Ther* 2008;24:309-319.

43. Nishino K, Fukushima A, Okamoto S, Ohashi Y, Fukata K, Ozaki A, et al. Suppression of experimental immune-mediated blepharocconjunctivitis in Brown Norway rats by topical application of FK506. *Graefes Arch Clin Exp Ophthalmol* 2002;240:137–143.

44. Pleyer U, Lutz S, Jusko WJ, Nguyen KD, Narawane M, Rückert D, et al. Ocular absorption of topically applied FK506 from liposomal and oil formulations in the rabbit eye. *Invest Ophthalmol Vis Sci* 1993;34:2737–2742.

45. Sasakawa Y, Sakuma S, Higashi Y, Sasakawa T, Amaya T, Goto T. FK506 suppresses neutrophil chemoattractant production by peripheral blood mononuclear cells. *Eur J Pharmacol* 2000;403:281–288.

46. Reitamo S, Van Leent EJ, Ho V, Harper J, Ruzicka T, Kalimo K, et al. Efficacy and safety of tacrolimus ointment compared with that of hydrocortisone acetate ointment in children with atopic dermatitis. *J Allergy Clin Immunol* 2002;109:539–546.

47. Reitamo S, Rustin M, Ruzicka T, Cambazard F, Kalimo K, Friedmann PS, et al. Efficacy and safety of tacrolimus ointment compared with that of hydrocortisone butyrate ointment in adult patients with atopic dermatitis. *J Allergy Clin Immunol* 2002;109:547–555.

48. Reitamo S, Remitz A, Kyllönen H, Saarikko J. Topical noncorticosteroid immunomodulation in the treatment of atopic dermatitis. *Am J Clin Dermatol* 2002;3:381–388.

49. Aoki S, Mizote H, Minamoto A, Suzuki M, Mishima HK, Tanaka H. Systemic FK506 improved tear secretion in dry eye associated with chronic graft versus host disease. *Br J Ophthalmol* 2005;89:243-244.

50. Ryu EH, Kim JM, Laddha PM, Chung ES, Chung TY. Therapeutic effect of 0.03% tacrolimus for ocular graft versus host disease and vernal keratoconjunctivitis. *Korean J Ophthalmol* 2012;26:241-247.

51. Tam PM, Young AL, Cheng AL, Lam PT. Topical 0.03% tacrolimus ointment in the management of
ocular surface inflammation in chronic GVHD. *Bone Marrow Transplant* 2010;45:957-958.

52. Moscovici BK, Holzchuh R, Chiacchio BB, Santo RM, Shimazaki J, Hida RY. Clinical treatment of dry eye using 0.03% tacrolimus eye drops. *Cornea* 2012;31:945-949.

53. Comstock T, DeCory H. Advances in corticosteroid therapy for ocular inflammation: loteprednol etabonate. *Int J Inflamm* 2012;2012:789623.

54. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoid 53. Comstock T, DeCory H. Advances in corticosteroid action: what is important? *Thorax* 2000;55:603–613.

55. Newton R. Molecular mechanisms of glucocorticoid action: what is important? *Thorax* 2000;55:603–613.

56. Stahn C, Buttgereit F. Genomic and nongenomic effects of glucocorticoids. *Nat Clin Pract Rheumatol* 2008;4:525–533.

57. Marsh P, Pflugfelder SC. Topical nonpreserved methylprednisolone therapy of keratoconjunctivitis sicca in sjogren’s syndrome. *Ophthalmology* 1999;106:811–816.

58. Pflugfelder SC, Maskin SL, Anderson B, Chodosh J, Holland EJ, De Paiva CS, et al. A randomized, double-masked, placebo-controlled, multicenter comparison of loteprednol etabonate ophthalmic suspension, 0.5%, and placebo for treatment of keratoconjunctivitis sicca in patients with delayed tear clearance. *Am J Ophthalmol* 2004;138:444–457.

59. McGhee CN, Dean S, Danesh-Meyer H. Locally administered ocular corticosteroids: benefits and risks. *Drug Saf* 2002;25:33–55.

60. Solomon A, Rosenblatt M, Li D, Monroy D, Ji Z, Lokeshwar BL, et al. Doxycycline inhibition of interleukin-1 in the cornea epithelium. *Am J Ophthalmol* 2000;130:688.

61. De Paiva CS, Corrales RM, Villarreal AL, Farley WJ, Li DQ, Stern ME, et al. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. *Exp Eye Res* 2006;83:526-535.

62. De Paiva CS, Corrales RM, Villarreal AL, Farley W, Li DQ, Stern ME, et al. Apical corneal barrier disruption in experimental murine dry eye is abrogated by methylprednisolone and doxycycline. *Invest Ophthalmol Vis Sci* 2006;47:2847-2856.

63. Nikodemova M, Watters JJ, Jackson SJ, Yang SK, Duncan ID. Minocycline downregulates MHC II expression in macroglia and macrophages through inhibition of IRF-1 and protein kinase C (PKC) alpha/betaII. *J Biol Chem* 2007;282:15208-15216.

64. Frucht-Pery J, Sagi E, Hemo I, Ever-Hadani P. Efficacy of doxycycline and tetracycline in ocular rosacea. *Am J Ophthalmol* 1993;116:88-92.

65. Zengin N, Tol H, Gündüz K, Okudan S, Balevi S, Endoğru H. Meibomian gland dysfunction and tear film abnormalities in rosacea. *Cornea* 1995;13:144-146.

66. Akpek EK, Merchant A, Pinar V, Foster CS. Ocular rosacea: patient characteristics and follow-up. *Ophthalmology* 1997;104:1863-1867.

67. Dursun D, Kim MC, Solomon A, Pflugfelder SC. Treatment of recalcitrant corneal epithelial erosions with inhibitors of matrix metalloproteinases-9, doxycycline and corticosteroids. *Am J Ophthalmol* 2001;132:8-13.

68. Hope-Ross MW, Chell PB, Kervick GN, McDonnell PJ, Jones HS. Oral tetracycline in the treatment of recurrent corneal erosions. *Eye (Lond)* 1994;8:384-388.

69. Liou LB. Serum and in vitro production of IL-1 receptor antagonist correlate with C-reactive protein levels in newly diagnosed, untreated lupus patients. *Clin Exp Rheumatol* 2001;19:515-523.

70. Ji H, Pettit A, Ohmura K, Ortiz-Lopez A, Duchatelle V, Degott C, et al. Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis. *J Exp Med* 2002;196:77-85.

71. Paramo JA, Orbe J, Fernandez J. Fibrinolysis/ proteolysis balance instable angina pectoris in relation to angiographic findings. *Thorax Haemost* 2001;86:636-639.

72. Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE. Beneficial effect on artificial tears made with autologous serum in patients with Keratoconjunctivitis sicca. *Arthritis Rheum* 1984;27:459-61.

73. Kono I, Kono K, Narushima K, Akama T, Suzuki H, Yamane K, et al. Beneficial effect of the local application of plasma fibronectin and autologous serum in patients with Keratoconjunctivitis sicca. *Arthritis Rheum* 1986;29:339-343.

74. Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I, Shimamura S., et al. Treatment of dry eye by autologous serum application in Sjogren’s syndrome. *Br J Ophthalmol* 1999;83:390-395.

75. Tananuvat N, Daniell M, Sullivan LJ, Yi Q, McKelvie V, Degott C, et al. Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis. *J Exp Med* 2002;196:77-85.

76. Tsunoda K, Goto E, Fujita H, Ono M, Inoue H, Saito I, Shimamura S., et al. Treatment of dry eye by autologous serum application in Sjogren’s syndrome. *Br J Ophthalmol* 1999;83:390-395.

77. Yoon KC, Jeong IY, Im SK, et al. Therapeutic effect of umbilical cord serum for the treatment of dry eye using 0.03% tacrolimus eye drops. *Ophthalmology* 2001;108:811-816.

78. Hope-Ross MW, Chell PB, Kervick GN, McDonnell PJ, Jones HS. Oral tetracycline in the treatment of recurrent corneal erosions. *Eye (Lond)* 1994;8:384-388.

79. Liou LB. Serum and in vitro production of IL-1 receptor antagonist correlate with C-reactive protein levels in newly diagnosed, untreated lupus patients. *Clin Exp Rheumatol* 2001;19:515-523.

80. Ji H, Pettit A, Ohmura K, Ortiz-Lopez A, Duchatelle V, Degott C, et al. Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis. *J Exp Med* 2002;196:77-85.

81. Paramo JA, Orbe J, Fernandez J. Fibrinolysis/ proteolysis balance instable angina pectoris in relation to angiographic findings. *Thorax Haemost* 2001;86:636-639.
eye associated with graft-versus-host disease. Bone Marrow Transplant 2007;39:231-235.

78. Na KS, Kim MS. Allogenic serum eye drops for the treatment of dry eye patients with chronic graft-versus-host disease. J Ocul Pharmacol Ther 2012;28:479-483.

79. Gabay C, Porter B, Fantuzzi G, Arend WP. Mouse IL-1 receptor antagonist isoforms: complementary DNA cloning and protein expression of intracellular isoform and tissue distribution of secreted and intracellular IL-1 receptor antagonist in vivo. J Immunol 1997;159:5905–5913.

80. Okanobo A, Chauhan SK, Dastjerdi MH, Kodati S, Dana R. Efficacy of topical blockade of interleukin-1 in experimental dry eye disease. Am J Ophthalmol 2012;154:63-71.

81. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 2008;8:349-361.

82. Li N, He J, Schwartz CE, Gjorstrup P, Bazan HE. Resolvin E1 improves tear production and decreases inflammation in a dry eye mouse model. J Ocul Pharmacol Ther 2010;26:431-439.

83. de Paiva CS, Schwartz CE, Gjörstrup P, Pflugfelder SC. Resolvin E1 (RX-10001) reduces corneal epithelial barrier disruption and protects against goblet cell loss in a murine model of dry eye. Cornea 2012;31:1299-1303.

84. Cortina MS, Bazan HE. Docosahexaenoic acid, protectins and dry eye. Curr Opin Clin Nutr Metab Care 2011;14:132-137.

85. Tylaska LA, Boring L, Weng W, Aiello R, Charo IF, Rollins BJ, et al. CCR2 regulates the level of MCP-1/CCL2 in vitro and at inflammatory sites and controls T cell activation in response to alloantigen. Cytokine 2002;18:184-190.

86. Oshima T, Sonoda KH, Tsutsumi-Miyahara C, Qiao H, Hisatomi T, Nakao S, et al. Analysis of corneal inflammation induced by cauterisation in CCR2 and MCP-1 knockout mice. Br J Ophthalmol 2006;90:218-222.

87. Goyal S, Chauhan S, Zhang Q, Dana R. Amelioration of murine dry eye disease by topical antagonist to chemokine receptor 2. Arch Ophthalmol 2009;127:882-887.

88. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinase in immune cell signaling. Immunol Rev 2009; 228:273-287.

89. Meyer DM, Jesson MI, Li X, Elrick MM, Funcakes-Shippy CL, et al. Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550 in rat adjuvant-induced arthritis. J Inflamm (Lond) 2010;7:41.

90. Ghoreschi K, Jesson MI, Li X, Lee JL, Ghosh S, Alsup JW, et al. Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). J Immunol 2011;186:4234-4343.

91. Liew SH, Nichols KK, Klamerus KJ, Li JZ, Zhang M, Fouls GN. Tofacitinib (CP-690,550), a Janus kinase inhibitor for dry eye disease: results from a Phase 1/2 trial. Ophthalmology 2012;119: 1328-1335.

92. Huang JF, Yafawi R, Zhang M, McDowell M, Rittenhouse KD, Sace F, et al. Immunomodulatory effect of the topical ophthalmic Janus kinase inhibitor tofacitinib (CP-690,550) in patients with dry eye disease. Ophthalmology 2012;119:e43-50.

93. Murphy CJ, Bentley E, Miller PE, McIntyre K, Leatherberry G, Dubielzig R, et al. The pharmacologic assessment of a novel lymphocyte function-associated antigen-1 antagonist (SAR 1118) for the treatment of keratoconjunctivitis sicca in dogs. Invest Ophthalmol Vis Sci 2011;52:3174-3180.

94. Semba CP, Torkildsen GL, Lonsdale JD, McLaurin EB, Geffin JA, Mundorf TK, et al. A Phase 2 randomized, double-masked, placebo-controlled study of a novel integrin antagonist (SAR 1118) for the treatment of dry eye. Am J Ophthalmol 2012;153:1050-1060.

95. Cavet M, Harrington K, Ward K, Zhang JZ. Mapracorat, a novel selective glucocorticoid receptor agonist, inhibits hyperosmolar-induced cytokine release and MAPK pathways in human corneal epithelial cells. Mol Vis 2010;16:1791-1800.

96. Shafiee A, Bucolo C, Budzynski E, Ward KW, López FJ. In vivo ocular efficacy profile of mapracorat, a novel selective glucocorticoid receptor agonist, in rabbit models of ocular disease. Invest Ophthalmol Vis Sci 2011;52:1422-1430.