

Matrix metalloproteinase-9 in the pathophysiology and diagnosis of dry eye syndrome

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Abstract: Dry eye and tear dysfunction are common ocular disorders that cause cornea barrier disruption resulting in a poorly lubricated and irregular cornea epithelium, eye irritation and blurred vision. Increased levels and activities of matrix metalloproteinases (MMPs), particularly MMP-9, have been detected in the tears and ocular surface epithelial and inflammatory cells in dry eye. MMPs have been found to participate in disruption of tight junctions in the apical cornea epithelium leading to their accelerated desquamation and barrier disruption. This review summarizes evidence showing the contribution of MMPs to dry eye pathogenesis and their roles as biomarkers and therapeutic targets.

Keywords: matrix metalloproteinase, dry eye, keratitis sicca, cornea, barrier function, Sjögren syndrome

Dry eye overview

Dry eye and tear dysfunction are common ocular disorders that cause cornea barrier disruption resulting in a poorly lubricated and irregular cornea epithelium, eye irritation and blurred vision. It affects millions of people worldwide and is one of the most frequent conditions for which patients seek eye care.¹ Aging and female sex are significant risk factors for dry eye which increases in prevalence around the fifth decade, with further increase every decade thereafter.^{2–13} Prevalence of dry eye varies from 2% to 50%, depending on the population studied and the diagnostic criteria for dry eye (symptom questionnaire vs. objective signs).^{1–3,6,7,9–11,14–23} There is scarce information about the natural history of dry eye, but there is a recognized disconnection between signs and symptoms; patients tend to be more symptomatic at early stages.^{24,25} Dry eye causes corneal irregularity^{26–28} and decreases functional vision by altering contrast sensitivity^{29–31} and, therefore, decreases quality of life with a significant burden on the individual as well as the society.^{23,32–35} A meta-analysis of 22 published studies showed increased odds ratio for depression and anxiety in patients with ocular Sjögren syndrome (SS).³⁶ There is increased evidence that dry eye is an inflammatory disease, and this review will focus on matrix metalloproteinases (MMPs) and their role in the pathogenesis of dry eye.

Cornea barrier disruption is a feature of dry eye

As the principal lens of the eye, the cornea has unique features to maintain its transparency and smooth surface that include a lack of blood vessels and a multilayered stratified, non-cornifying epithelium. The differentiated apical epithelial cells produce

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heavily glycosylated transmembrane mucins (MUC1, MUC4 and MUC16) that associate with carbohydrate-binding proteins in tears, particularly galectin 3, to form a glycocalyx that maintains hydration and surface smoothness.³⁷ The cornea epithelium also serves as a barrier to adverse environmental conditions, microbial pathogens and immunogens/allergens. Paracellular barrier is maintained by tight junctions in the apical epithelium, while the transcellular barrier is provided by transmembrane mucins and their bound O-glycans in the glycocalyx.³⁷

MMPs have been found to perturb or disrupt the corneal epithelial barrier by cleaving a variety of substrate molecules involved in barrier maintenance, including occludin (MMP-9), galectin 3 (MMP-2 and -9) and MUC16 (MMP-7).^{38–40} Gelatinases have been implicated in physiologic desquamation of the cornea epithelium. Decreased corneal epithelial desquamation in vitamin A-deficient rats was associated with significantly decreased MMP-2 and -9 expression.⁴¹ A study showed clusters of disrupted tight junction proteins, occludin and claudin, in conjunction with increased MMP-2 immunoreactivity in samples of superficial cornea epithelium obtained at night in *Xenopus laevis*.⁴² While this mechanism for corneal epithelial desquamation has not been confirmed in human corneas, increased levels of MMP-2 and -9 have been found in cornea epithelium and tear fluid obtained from eyes with recurrent corneal epithelial erosion,^{43,44} and increased pro- and active forms of MMP-9 have also been found in tears sampled from the closed eye during sleep when recurrent erosions typically occur while tear production and clearance are decreased.⁴⁵

There is mounting evidence that MMPs contribute to the altered cornea barrier function that is observed in dry eye.^{46–48} Increased cornea epithelial permeability to fluorescent dyes is observed in human dry eye (sodium fluorescein) and experimental mouse models (fluorescent dextran conjugates).^{48,49} Exposure to desiccating stress increased MMP-9 expression and activity in the corneal epithelium and was found to lyse the tight junction protein occludin in the apical corneal epithelium, leading to increased permeability to the tracer molecule 70 kDa Oregon Green dextran in mice.^{48,50,51} An important component of the glycocalyx, galectin 3, is an MMP-9 substrate.^{39,52} MMP-7 has been reported to cleave MUC16, the principal mucin constituent of the cornea epithelial glycocalyx, from cultured human cornea epithelial cell membranes.⁴⁰ Taken together, these findings implicate MMPs in the disruption of molecules maintaining para- and transcellular cornea barrier.

Cornea epitheliopathy in dry eye

Among the most exposed mucosal surfaces in the body, the cornea and conjunctiva depend on tear production by the lacrimal functional unit to maintain hydration, smoothness and optical clarity.⁵³ Among the myriad of proteins in the tears are anti-inflammatory molecules, including cytokine antagonists (e.g., interleukin [IL]-1RA), tissue inhibitors of MMPs (TIMPs) and clusterin (CLU).^{47,54–59} Disease or dysfunction of the lacrimal functional unit alters the balance of MMPs and their inhibitors and stimulates activation of innate inflammatory pathways in the ocular surface epithelium and resident immune/inflammatory cells. A key stimulus is increased tear osmolarity, primarily due to increased tear sodium ion concentration.^{60,61} Osmotic stress has been found to activate cell signaling pathways, such as the mitogen-activated protein kinases (MAPK) and the nuclear factor kappa B (NF- κ B) pathways, that stimulate production of MMP-9 and its physiologic activator (e.g., MMP-3^{47,62,63}), as well as inflammatory cytokines such as IL-1 β that also stimulate MMP production.^{62,64,65} Lacrimal dysfunction also results in reduced tear concentrations of TIMPs and CLU.^{56,66}

Increased MMP-9 activity in the epithelium and tears promotes lysis of tight junction proteins and accelerates detachment of apical corneal epithelial cells, exposing less-mature subapical epithelial cells that lack a developed glycocalyx.³⁸ The irregular cornea surface with disrupted permeability barrier in dry eye reduces visual performance, causes irritation due to decreased surface lubrication and increases blink frequency that can exacerbate inflammation and protease activity (Figure 1).

Correlations between MMP-9 and severity

Tear MMP-9 level or activity has been evaluated in tear samples from normal subjects and dry eye patients using a variety of methods (Table 1). Using a point-of-care lateral flow immunoassay (InflammaDry[®]; Rapid Pathogen Screening, Sarasota, FL, USA), which detects both pro- and active MMP-9 at tear concentrations ≥ 40 ng/mL, elevated tear MMP-9 was found to have high sensitivity and specificity with clinical diagnosis of dry eye in two studies, and in another study, a positive result was significantly correlated with severity of irritation symptoms and ocular surface dye staining, tear break-up time and systemic autoimmune disease, particularly SS.^{67–69} Using the same assay, Lanza et al found only 39% of subjects with symptoms of dry eye had a positive MMP-9 assay and there was no difference in the profile of signs and symptoms between MMP-9-positive and

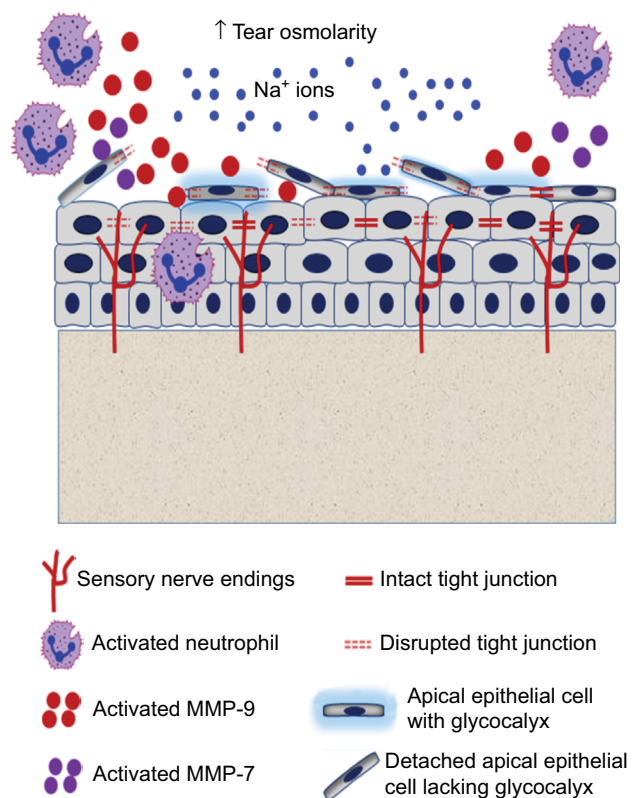


Figure 1 Desiccation increases the tear osmolarity and the concentrations of inflammatory cytokines in the tears and stimulates the ocular surface epithelial cells and neutrophils to produce and secrete latent MMPs.

Notes: These MMPs, once activated by other proteases, digest substrates on the ocular surface. These include tight junction proteins, such as occludin, in the apical epithelial cells and galectin-3, a key component of the glycocalyx. Loss of tight junctions leads to accelerated epithelial desquamation, cornea barrier disruption and exposure of corneal nociceptors which results in cornea pain. Activated MMP-7 has been found to lyse the membrane mucin MUC16 that is a key component of the corneal epithelial glycocalyx.

Abbreviation: MMPs, matrix metalloproteinases.

-negative subjects.⁷⁰ Using a quantitative immunobead assay, tear MMP-9 concentration was directly correlated to tear osmolarity and inversely correlated to Schirmer test scores that are a measure of tear production.⁷¹ In a study measuring tear MMP-9 activity rather than concentration, MMP-9 activity increased with categorical severity of tear dysfunction using the Dry Eye Workshop (DEWS) criteria and tear MMP-9 activities showed significant positive correlation with symptom severity scores, corneal and conjunctival fluorescein staining, topographic surface regularity index and percentage area of abnormal superficial corneal epithelia measured by confocal microscopy, and inverse correlation with low-contrast visual acuity and fluorescein tear break-up time. *MMP-9* gene expression and immunoreactivity were also found elevated in conjunctival epithelial cells obtained from normal subjects and patients with SS-associated dry eye and meibomian gland disease (MGD) that also causes tear instability.⁷² Levels of MMP-9 transcripts were significantly higher in both types of tear dysfunction than asymptomatic

controls, and MMP-9 expression was higher in SS than MGD. MMP-9 expression significantly correlated with clinical severity in the SS group. Additionally, corticosteroid treatment significantly reduced MMP-9 expression in both groups.

Significantly higher concentration of MMP-9 was found in eyes with conjunctivochalasis (a condition of conjunctival redundancy that alters tear distribution, clearance and stability), and active MMP-9 was the most abundant form in tear samples from these patients. MMP-9 significantly decreased after surgical resection of the redundant conjunctiva.⁷³ Increased MMP-8 and -9 concentrations and decreased TIMP-1 concentration were also reported in tears from patients with the fibrotic ocular surface inflammatory diseases, Stevens–Johnson syndrome (SJS) and ocular cicatricial pemphigoid (OCP), with the highest MMP-9 concentration in SJS. MMP-8/TIMP-1 and MMP-9/TIMP-1 ratios were markedly elevated in both SJS and OCP groups (SJS>OCP) compared to controls.⁷⁴ Across all study groups, MMP-9 was strongly correlated with MMP-8 and myeloperoxidase levels, suggesting that neutrophils are the primary source of the tear MMP-9 in these conditions.

In addition to the association between MMPs and ocular surface diseases, Aluri et al found significantly higher protein expression and activities of MMP-2 and -9 in the lacrimal glands of two mouse models of SS (MRL/lpr and NOR/LtJ strains) compared to wild-type strains.⁷⁵ MMP-2/-9 inhibitor peptide treatment of MRL/lpr mice improved aqueous tear production and decreased the number and size of lymphocytic foci in the inflamed lacrimal glands.⁷⁵

Effects of MMP-9 gene deletion or inhibition on corneal epithelial disease in mouse models

The role of MMP-9 in dry eye was further confirmed using gene knock-out (KO) and inhibitors in murine models. MMP-9 was found to disrupt corneal epithelial tight junction proteins both in vitro and in vivo and participate in acute corneal barrier disruption in the desiccating stress dry eye model.^{38,47,48,63,76,77} MMP-9KO was noted to be resistant to dry eye-induced corneal changes, but exogenous administration of recombinant MMP-9 recapitulated the acute corneal barrier changes observed with wild-type mice.

Combined alkali burn and dry eye

Corneal alkali burns are devastating injuries with potentially blinding consequences, where MMPs have been implicated.^{78–81} Because the high-airflow, low-humidity controlled environments that we all live and work in are an

Table 1 MMP-9 in dry eye

Authors	Sample/method of MMP-9 detection	Findings
Acera et al ⁷³	ELISA, zymography	MMP-9 concentration was significantly increased in eyes with conjunctivochalasis with and without dry eye; MMP-9 was activated in these eyes
Arafat et al ⁷⁴	Multiplex immunobead assay	Increased MMP-8 and -9 and decreased TIMP1 concentration in tears from SJS and OCP (SJS>OCP)
Aragona et al ⁷²	RT-PCR and immunostaining	MMP-9 transcripts were significantly higher in conjunctiva from SS and MGD (SS>MGD) than asymptomatic controls. MMP-9 expression significantly correlated with clinical severity in SS. Corticosteroid significantly reduced MMP-9 expression
Chotikavanich et al	Tears/enzyme activity assay Conjunctival epithelium/mRNA by RT-PCR	Progressive increase in tear activity with severity and significant positive correlation with irritation symptoms and corneal disease; conjunctival gene transcripts significantly increased
Lanza et al ⁷⁰	Tears/lateral flow immunoassay	39% positive in symptomatic patients, no difference in profile of dry eye signs and symptoms between MMP-9-positive and -negative subjects
Messmer et al ⁶⁹	Tears/lateral flow immunoassay	40% of dry eye patients MMP-9 positive; significant correlation between MMP-9 and irritation symptoms, tear break-up time, ocular surface dye staining and systemic autoimmune disease, particularly SS
Sambursky et al ⁶⁸	Tears/lateral flow immunoassay	Sensitivity of 85% and sensitivity of 94% for dry eye diagnosis, compared to clinical assessment
Sambursky et al ⁶⁷	Tears/lateral flow immunoassay	Sensitivity of 81% and sensitivity of 98% for dry eye diagnosis based on signs and symptoms; sensitivity of 86% and sensitivity of 97% for dry eye diagnosis based on signs only
VanDerMeid et al ⁷¹	Tears/immunobead assay	Among five MMPs (1, 2, 7, 9 and 10), MMP-7 and -9 had the highest concentration; MMP-9 was inversely correlated with tear production and positively correlated with tear osmolality

Abbreviations: ELISA, enzyme-linked immunosorbent assay; MGD, meibomian gland disease; MMPs, matrix metalloproteinases; OCP, ocular cicatricial pemphigoid; mRNA, messenger RNA; RT-PCR, reverse transcriptase polymerase chain reaction; SJS, Stevens–Johnson syndrome; SS, Sjögren syndrome; TIMP, tissue inhibitors of MMPs.

underrecognized source of desiccation, we created a more severe model of alkali burn by subjecting mice to desiccating stress immediately after creation of the corneal alkali burn.^{82–85} This simulated the most severe burn cases, where extensive facial and eyelid thermal injury results in eye exposure and desiccation due to loss of protection from the eyelids and reduced blinking. We found there was an additive pathogenic effect of dry eye to alkali burn, with amplification of MMP-3, -8, -9 and -12, IL-6 and IL-1 β mRNA expression and sterile corneal perforation in ~30% of animals subjected to the chemical burn and desiccating conditions.⁸² There was a significant tenfold increase in MMP-9 mRNA levels and a twofold increase in MMP-9 activity in alkali burn plus desiccation corneas, compared to the dry eye alone group.⁸² These results demonstrate that MMPs contribute to the desiccation-induced worsening of cornea disease in conditions of severe inflammation.

MMP-9 as a diagnostic biomarker

InflammaDry is a rapid (<10 min) innovative immunoassay test to measure MMP-9 tear concentration in tears, which was designed to be used in the office by medical doctors, nurse or ancillary staff. Tear samples are collected from the inferior meniscus and palpebral conjunctival by dabbing the surface

with a collecting fleece that is assembled with the test cassette after adding the assay buffer. The test is positive (meaning MMP-9 concentration is ≥ 40 ng/mL) when two lines (one blue and one pink) are visualized in the test window. A blue line indicates that the tear concentrations are <40 ng/mL. The advantages of this assay are its ease of use and the rapid time to obtain results. Because it is nonquantitative, patients with high MMP-9 levels will have the same test result as those with moderately high levels, as once the threshold concentration of MMP-9 is detected, the pink line will be present. When Sambursky et al evaluated 206 patients with dry eye, they reported a sensitivity of 85%, specificity of 94%, negative predictive value of 73% and positive predictive value of 97% using the InflammaDry test.⁶⁸

The clinical trials summarized in Table 1 show conflicting results regarding the diagnostic utility of the InflammaDry tear MMP-9 immunoassay. This may be due to the design of this test that detects a threshold MMP-9 concentration. Since correlation has been found between level of MMP-9 gene expression and tear activity and clinical severity of dry eye disease (DED), it is possible that patients with mild disease may have tear MMP-9 concentrations above normal levels, but below this threshold level. This points to the need for a quantitative assay that measures MMP-9 protein, preferably

latent and activated forms, or MMP-9 activity. None of the commercially available assays that have been used in reported clinical trials are currently approved for clinical diagnosis, but results of these studies show the potential of these technologies.

Therapeutic effects of MMP-9 inhibitors on cornea disease of dry eye

There is no specific MMP-9 inhibitor with an approved indication to treat dry eye. However, anti-inflammatory therapy with doxycycline, azithromycin or the corticosteroid dexamethasone has shown promising results in preclinical studies and in human trials as off-label drugs, and the Food and Drug Administration (FDA)-approved dry eye therapy cyclosporine A (CsA) emulsion has been found to inhibit MMP production. All of these agents are currently used to treat dry eye, and their efficacy in improving the corneal epithelial disease of dry eye is presented in more detail below. To the best of the authors' knowledge, there have been no clinical trials investigating the efficacy of selective MMP-9 inhibitors in dry eye.

Doxycycline and azithromycin

Doxycycline

Doxycycline, a tetracycline antibiotic with potent anti-inflammatory properties in subantimicrobial doses, has been shown to inhibit MMPs *in vitro*.^{86–91} Doxycycline has been found to decrease activation of the MAPK and NF- κ B pathways and improve corneal barrier function and the number of mucus-producing conjunctival goblet cells in dry eye.^{48,62,65,92–95} We and others have tested different preparations of doxycycline in our mouse desiccating stress dry eye model, and we have shown significant improvement in corneal barrier function (reduced uptake of a fluorescent dye) and decreased transcript levels of inflammatory cytokines such as IL-1 β , IL-6, tumor necrosis factor- α and MMPs.^{48,50,77,92,96,97} Similar results were obtained with a variety of formulations, including topically applied doxycycline dissolved in saline or hydroxypropyl methylcellulose, glycerin and excipients, or subconjunctival injections of doxycycline nanoparticles.^{77,96,97} We also observed that topical administration of doxycycline decreased apical corneal epithelial desquamation that is accelerated in desiccating stress and it preserved corneal normal architecture.⁹⁶ Doxycycline increased the rate of corneal epithelial wound healing and decreased MMP-13 and -9 mRNA transcripts, MMP-9 activity and immunoreactivity in the combined model of alkali burn and dry eye.⁹⁸ This was accompanied by a decrease in production of neutrophil

gelatinase-associated lipocalin (NGAL)–MMP-9 formation and lower levels of IL-1 β .

The greatest challenge with topical delivery of doxycycline is its rapid oxidation when exposed to environmental oxygen, which limits its stability and potency. Oral doxycycline has also been used off-label to treat recurrent corneal erosions, ocular rosacea and MGD,^{99–102} as well as other inflammatory diseases where MMPs are implicated, including rheumatoid arthritis, and atherosclerotic cardiovascular disease.¹⁰³ It is FDA approved to treat periodontitis, a disease of the gums where increased levels of MMPs are found. However, side effects of oral administration, such as gastrointestinal upset, photosensitivity and vaginal candidiasis, can occur.

Azithromycin

Azithromycin is a macrolide antibiotic that is used for treating a variety of bacterial and chlamydial infections. Like doxycycline, it is also recognized as having anti-inflammatory effects by suppressing cytokine and MMP-9 production by airway epithelial cells. Azithromycin is currently used orally and topically to treat ocular rosacea and MGD. Azithromycin inhibited production of inflammatory cytokines, chemokines and MMP-1, -3 and -9 in cultured human corneal epithelial cells (HCEC) stimulated by the toll like receptor 2 (TLR2) ligand, zymosan.¹⁰⁴ Azithromycin inhibited production of all of these MMPs in a dose-dependent fashion, showing greater reduction in MMP-9 production by zymosan-stimulated HCEC than anti-TLR2 antibody or an NF- κ B inhibitor. The effects of 4 weeks of topical azithromycin 1% therapy were evaluated on the mRNA expression of a variety of inflammatory mediators, including MMP-9, in conjunctival epithelial cells of patients with MGD.¹⁰⁵ MMP-9 activity was also measured in the tears. At baseline prior to initiating therapy, levels of MMP-9 transcripts in the conjunctival epithelium were 13.5-fold higher in MGD samples than those from normal eyes. Following 4 weeks of azithromycin treatment, MMP-9 expression significantly decreased toward normal levels, although it rebounded toward pretreatment values 4 weeks after cessation of azithromycin. Change in tear MMP-9 activity was similar to the pattern observed for MMP-9 transcripts.

Osmoprotectants

As noted above, elevated tear osmolarity is a common feature of tear dysfunction. Osmotic stress is a potent inflammatory stimulus on the ocular surface epithelial cells, which is of similar magnitude to stimulation with lipopolysaccharide. Artificial tears are the first-line therapy of dry eye, and these

typically contain one or more lubricating polymers plus additives. Among the additives are molecules that protect against osmotic stress. The effects of three osmoprotectants (l-carnitine, erythritol, betaine) on inhibiting stimulated expression of collagenase MMP-13, gelatinases MMP-2 and -9, stromelysin MMP-3, and matrilysin MMP-7 in HCEC by hyperosmotic media were also investigated.¹⁰⁶ Osmotic stress significantly stimulated mRNA expression of all of these MMPs in an osmolarity-dependent fashion. l-Carnitine significantly suppressed the expression of all of the MMPs except MMP-2, and erythritol and betaine significantly suppressed MMP -2, -7 and -9. Gelatin zymography showed that all three osmoprotectants inhibited activation of MMP-2 and -9.

Corticosteroids

Dexamethasone, a prototype corticosteroid, has been used topically in murine models of dry eye with great success. We have shown that its topical administration four times per day during experimental exposure to desiccating stress decreases MAPKs (notably the june amino-terminal kinase [JNK] pathway); blunts the production and activity of MMPs and also inflammatory cytokines and chemokines, compared to saline control.⁷⁷ Also, in vitro, dexamethasone has been shown to inhibit the interferon- γ -induced endoplasmic reticulum stress and unfolded protein response in cultured conjunctival goblet cells, preserving mucin production.¹⁰⁷ A biodegradable dexamethasone-loaded nanowafer used once a day on alternate days in the desiccating stress model showed similar or better control of inflammation and corneal barrier disruption, compared to topically administered dexamethasone solution four times per day, demonstrating not just greater efficacy but also the potential for improved patient compliance due to the reduced dosing frequency.¹⁰⁸ Application of topical 0.1% dexamethasone four times per day showed a positive effect in the combined model of alkali burn and desiccating stress, by significantly decreasing mRNA and protein levels of MMP-1, -3, -9 and -13, decreasing MMP-9 gelatinolytic activity and NGAL-MMP-9 complex formation, while causing an impressive improvement in corneal opacification and preventing corneal perforation, compared to saline controls.⁹⁸ Studies with gene KO and pharmacologic inhibitors showed that part of the beneficial effects from dexamethasone in this model were through an increase in MMP-8.⁸⁴

Nonpreserved topical methylprednisolone administered three to four times per day significantly improved signs and symptoms of dry eye in patients with SS.¹⁰⁹ Clinically, prolonged use of topical corticosteroids is plagued by frequent side effects, such as cataract induction and glaucoma, that

limit their wide use in dry eye;¹⁰⁹ however, the introduction of “soft-steroids” that carry a markedly reduced risk of sight-threatening side effects has led to community acceptance of corticosteroid therapy on a limited basis.^{110,111}

Topical treatment with 0.1% dexamethasone for 2 weeks prior to a low-humidity exposure stress using specially designed goggles significantly decreased corneal and conjunctival staining, improved blink rate and decreased Human leukocyte antigen - D related (HLA-DR) RNA levels in conjunctiva of patients with aqueous deficient dry eye.¹¹² Significant improvement in signs and symptoms of dry eye were observed after 3 weeks of topical 0.1% fluorometholone therapy in dry eye patients subjected to a 2-hour challenge in a controlled adverse environment exposure,¹¹³ which increases the concentrations of MMP-9 and other inflammatory markers in tears.¹¹⁴

CsA

CsA was first discovered in 1976 as a weak antimycotic agent produced by the soil fungus *Tolypocladium inflatum*, and it was subsequently found to have immunomodulatory properties that led to its widespread use as an immunosuppressant to prevent rejection of solid organ transplants.^{115,116} After topically applied CsA was found to be an effective treatment for canine keratoconjunctivitis, it was also noted to improve human DED and a 0.05% emulsion received FDA approval to treat dry eye-associated inflammation in 2002.^{117,118} Since then, the medication has been widely used to treat millions of dry eye patients, and a recent comprehensive review describes its efficacy in DED.¹¹⁹ CsA is a calcineurin inhibitor that impairs T cell activation and cytokine production. CsA not only strikingly increases conjunctival goblet cell (GC) numbers in humans and animal models of dry eye, but also decreases epithelial apoptosis.^{118,120–126} By doing so, it has an indirect effect on the ocular surface by increasing the production of immunomodulatory factors by conjunctival goblet cells.^{127,128} CsA has been shown to decrease MMP-2, -3, -9 and -13 production and activity in cultured human gingival and pterygium fibroblasts.^{129–131} Topical CsA treatment was found to improve thyroid orbitopathy-related dry eye symptoms and decreased MMP-9 expression in conjunctival epithelial cells obtained from patients with thyroid orbitopathy.¹³² Results from a retrospective study where patients were treated with 2,000–4,000 mg of oral omega-3 fatty acids and artificial tear replacement with and without CsA emulsion showed that 85% of patients using CsA reported at least 50% improvement in symptoms. About 50% of these showed conversion from a positive to negative InflammDry test, suggesting that CsA decreased MMP-9 concentration in the tears of these patients.¹³³

CLU

CLU is a glycoprotein produced and secreted by the mucosal epithelia; it is cytoprotective, anti-inflammatory and serves as an extracellular chaperone that inhibits the activity of MMPs, including MMP-2, -3, -7 and -9. CLU inhibited MMP-9-mediated lysis of epithelial tight junctions in the MCF-7 mammary epithelial cell line. Furthermore, the desiccating stress mouse model of dry eye depleted CLU in the ocular surface epithelium.¹³⁴ In a subsequent study from the same group, using the same mouse dry eye model, CLU prevented and reversed corneal epithelial barrier disruption by sealing the epithelial surface in a critical all-or-none concentration fashion.⁵⁶ When the CLU level was decreased below the critical all-or-none threshold, the cornea barrier again became vulnerable to desiccating stress. CLU was found to bind to the surface of desiccated corneas, and in vitro, to galectin 3, a key component of the corneal epithelial glycocalyx that maintains transcellular barrier function.

Conclusion

In summary, this review presents evidence from animal models and clinical trials that clearly supports a role for MMPs in the pathogenesis of dry eye-induced corneal disease and indicates that MMP-9 is a valid biomarker of ocular surface inflammation and severity of DED, as well as a disease-relevant therapeutic target.

Acknowledgments

This work was supported by NIH grant EY11915 (SCP), DoD award 5W81XWH-13-1-0146 (SCP), W81XWH-12-1-0616 (CSdP), NIH core grants (EY002520, EY020799 and CA125123), NIH training grant T32 AI053831 (FB), NIH funding to Cytometry and Cell Sorting Core at Baylor College of Medicine (NIAID P30AI036211, NCI P30CA125123 and NCRR S10RR024574), Biology of Inflammation Center Baylor College of Medicine, an unrestricted grant from Research to Prevent Blindness, New York, NY (SCP), the Oshman Foundation, Houston, TX (SCP), the William Stamps Farish Fund, Houston, TX (SCP), Hamill Foundation, Houston, TX (SCP) and Sid W Richardson Foundation, Ft Worth, TX (SCP).

Disclosure

The authors report no conflicts of interest in this work.

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