The Practical Detection of MMP-9 Diagnoses Ocular Surface Disease and May Help Prevent Its Complications

Herbert E. Kaufman, MD

**Purpose:** To evaluate the importance and practicality of testing for matrix metalloproteinase 9 (MMP-9) in dry eye and ocular surface disease. This enzyme, which can cause tissue damage, seems also to be the most reliable diagnostic indicator of ocular surface disease.

**Methods:** Enzyme-linked immunosorbent assay, polymerase chain reaction, diffusion, and InflammaDry, a new rapid immunoassay by RPS (Rapid Pathogen Screening Inc).

**Results:** MMP-9 measurement is sensitive and accurate for diagnosing dry eye and ocular surface disease and compares favorably in both sensitivity and specificity against the existing methods of dry eye diagnosis. Abnormal elevations of MMP-9 may predict post-laser in situ keratomileusis complications and refractive complications such as epithelial ingrowth and corneal ulceration. The presence of elevated MMP-9 on the ocular surface will identify those patients who should receive antiinflammatory therapy, such as cyclosporine, and may predict those patients who will respond to this therapy.

**Conclusions:** A rapid in-office test that is sensitive for identifying inflammatory dry eye and ocular surface disease may facilitate better preoperative management of the ocular surface. Optimization of the ocular surface perioperatively would be expected to reduce complications from laser in situ keratomileusis and other surgeries that often make the underlying disease worse. This test may also indicate the need for antiinflammatory therapies, such as cyclosporine or steroids, and also may predict those patients who are more likely to respond.

**Key Words:** matrix metalloproteinases, keratitis sicca, ocular surgery, inflammation, nano-detection, LASIK

In 1969, Dohlman and his co-workers identified metalloproteinases as substances produced in ocular surface diseases that were important contributors to corneal ulceration, destruction, and perforation.1–3 Although there are more than a dozen matrix metalloproteinases (MMPs) that can dissolve collagen and other tissues (Table 1), this review is primarily concerned with MMP-9 (also called gelatinase-B). Although numerous important articles have documented the role of these metalloproteinases in ocular surface disease, both as a marker of the disease and as an agent of their pathology, they are being reviewed now because, for the first time, a new in-office immunoassay for the detection of MMP-9 is available. This can be of great value for both the detection of ocular surface disease and the prevention of complications after laser in situ keratomileusis (LASIK) and other ophthalmic surgeries.4

**KERATITIS SICCA**

Keratitis sicca is a complex disease with various components of aqueous deficiency from the lacrimal and accessory lacrimal glands, meibomian gland deficiency, and abnormal lipids stimulating evaporation and inflammation of the eyelids. It is usually referred to as dysfunctional tear syndrome (DTS), dry eye, and keratitis sicca. It occurs, in part, as a function of aging and atrophy of the accessory and lacrimal glands; as Sjogren syndrome, an immunomodulated disease; as a function of other autoimmune diseases (virtually all autoimmune diseases, such as lupus, graft-versus-host disease, rheumatoid arthritis, Stevens–Johnson syndrome, toxic epidermal necrolysis, and other skin diseases are associated with keratitis sicca); and can be exacerbated by a variety of common drugs, such as antihistamines, antihypertensives, and antidepressants. In its severe form, DTS can lead not only to disruptions of vision and corneal ulceration but also to corneal perforation and blindness. For clinicians, two potentially separate aspects of ocular surface disease are of primary concern, first, making the diagnosis of ocular surface disease,5 and second, determining the optimal therapy and monitoring its efficacy.6 In ocular surface disease associated with inflammation, where the degree of inflammation may be demonstrated by elevated tear levels of MMP-9, clinicians can better predict those patients who will respond to antiinflammatory therapy such as cyclosporine.7

The precise diagnosis of dry eyes has been clinically problematic. The Schirmer tear test, although extremely useful, sometimes correlates poorly with the patient’s symptoms and the other clinical appearances of dry eyes. Tear break-up time, similarly, is useful, but often correlates poorly with the clinically observable phenomena and patient signs and symptoms.8–11 It is thought that hyperosmolarity may be the gold standard for the diagnosis of dry eyes, but current test results have been variable12 and show sensitivity and specificity that range from 64% and 71%,13 respectively, to 73% and 92%.14 Moreover, tests are expensive and it has been difficult to collect tears without some stimulation. As we consider this as clinicians and consider the possibility of different therapies, it is possible that hyperosmolarity may be less important
than the presence or absence of ocular inflammation in the causation of signs and symptoms. Chotikavanich et al 15 showed that the MMP-9 level correlates with symptom severity scores in dry eye patients as well as with their decreased low-contrast visual acuity and tear break-up time. In fact, elevated levels of MMP-9 may be a more sensitive diagnostic marker than clinical signs.16

Early studies of MMP-9 involved relatively complex assays using the dissolution of gelatin or radiolabeled gelatin by the MMP.15 These and similar assays, which will be discussed later, were complex and suitable only for laboratory investigation as opposed to routine clinical diagnosis. Despite this, much very important work was done. For example, tears and saliva were initially tested in patients with Sjogren syndrome.17 In 1998, Konttinen et al 18 showed that a collagenase is present in the saliva of Sjogren patients and raised the question whether this gelatinase activity, which can degrade basement membrane collagens, was present and changed in the saliva of Sjogren patients. Subsequently, it was shown that when the tears and saliva of patients with Sjogren disease were compared with those of healthy controls, MMP-9 (as well as some other active MMPs and inflammatory inhibitors) was significantly elevated.19 It was suggested that this gelatinase enzyme could not only be an accurate marker for the disease but a factor in producing the inflammatory disease itself.19–22 More recently, the presence of MMP-9 in patients with pure keratitis sicca,15,22 or DTS was studied by Pfugfelder, showed that MMP-9 activity in unstimulated tear fluid was much higher than that in healthy controls and that the mRNA transcripts for MMP-9 were higher.

Nichols et al 8 showed that “although patient-reported symptoms are moderately repeatable from visit to visit, many of the procedures clinically used to diagnose and monitor dry eye syndromes are largely unrepeatable.” The clinical study by Chotikavanich et al 15 working with Pfugfelder concludes, “MMP-9 appears to be a potentially useful biomarker for diagnosing, classifying, and monitoring dysfunctional tear syndrome” (Fig. 1).22

The Pfugfelder group has done considerable laboratory work with mice using an evaporation-induced keratitis sicca.23,24 They found that this desiccating stress stimulates the production of MMP by the corneal epithelium.25,26

Corrales et al 25 working with Pfugfelder, showed that desiccating stress stimulates the expression of MMPs by the corneal epithelium in mice and that in a strain-dependent fashion, MMPs cause the disruption of the corneal barrier, thus increasing permeability (staining) and corneal irregularity.

**TABLE 1.** Classification of MMPs, Their Inhibitors, and Potential Inducers of Transcription

<table>
<thead>
<tr>
<th>Collagenases</th>
<th>Gelatinases</th>
<th>Stromelysins</th>
<th>Membrane-type MMPs</th>
<th>Matrilysin</th>
<th>Enamelysin</th>
<th>Metalloelastase</th>
<th>Others</th>
<th>Inhibitors</th>
<th>Potential Inducers of Transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>MMP-2</td>
<td>MMP-3</td>
<td>MMP-14</td>
<td>MMP-7</td>
<td>MMP-20</td>
<td>MMP-12</td>
<td>MMP-19</td>
<td>TIMP-1</td>
<td>BSG</td>
</tr>
<tr>
<td>MMP-8</td>
<td>MMP-9</td>
<td>MMP-10</td>
<td>MMP-15</td>
<td>MMP-26</td>
<td>MMP-21</td>
<td>MMP-22</td>
<td>MMP-21</td>
<td>TIMP-2</td>
<td>TCF-20</td>
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<td>MMP-13</td>
<td>MMP-11</td>
<td>MMP-16</td>
<td>MMP-17</td>
<td>MMP-24</td>
<td>MMP-27</td>
<td>MMP-28</td>
<td>MMP-27</td>
<td>TCF-20</td>
<td>TNF</td>
</tr>
</tbody>
</table>

BSG, basigin; TCF, transcription factor; TIMP, tissue inhibitors of matrix metalloproteinases; TNF, tumor necrosis factor.

**FIGURE 1.** MMP-9 activity in tear samples of normal subjects (NL), patients with meibomian gland disease (MGD), and Sjogren syndrome (SS).22 Reprinted with permission from Association for Research in Vision and Ophthalmology, 2001.
TREATMENT OF DRY EYES AND OTHER OCULAR SURFACE DISEASE

In the past, effective MMP inhibitors have been synthesized. One of them, Galardin, was tested clinically. It was found to delay corneal destruction after pseudomonas infection. It was then tested on 556 patients with a variety of corneal ulcers, and it significantly reduced corneal perforations in clinical trials. It is not totally clear why Galardin was never approved and commercialized, but at the time it was tested, it was not feasible to determine those patients who had elevated metalloproteinase levels and those who did not, nor was it possible to measure those patients who actually responded to the treatment in terms of proteinase inhibition. It seems likely that the results would have been far more dramatic had a convenient clinical assay been possible for patient selection and evaluation of therapy because some of the ulcers may have been chronic and no longer subjected to proteinase activity.

In a rat thermal injury model, it has been shown that MMP-9 delays corneal epithelial healing and reepithelialization is impeded by these products of the resident corneal epithelial cells, which destroy adhesive structures at the basement membrane zone. Failure to reepithelialize was found to correlate with an increase in the amounts of gelatinolytic MMPs present in the rat cornea; inhibition of that synthesis correlated with inhibition of basement membrane dissolution.

PRODUCTION OF MMP-9 AND INFLAMMATORY CYTOKINE EXPRESSION AND INHIBITION

Brignole et al showed that 6 months of treatment with topical cyclosporine A can reduce inflammatory markers in patients with keratitis sicca. Turner et al found a similar reduction in inflammatory markers after 6 months of treatment of patients with moderate to severe dry eyes with cyclosporine and corticosteroids can decrease inflammation as part of dry eye or other ocular surface disease. Because cyclosporine and corticosteroids have been shown to decrease production of inflammatory markers such as MMP-9, similar, doxycycline, which has been found especially useful in the treatment of meibomian gland disease, also decreases the production and effect of MMP-9. It has been postulated that inflammation activates a mitogen-activated protein kinase, and this in turn activates the production of the MMP-9. This pathway seems especially susceptible to doxycycline inhibition.

From the point of view of an ophthalmologist, the presence of elevated levels of MMP-9 indicates an active ocular surface inflammation as part of dry eye or other ocular surface disease. Because cyclosporine and corticosteroids have been shown to decrease production of inflammatory markers such as MMP-9 in human clinical trials, elevated levels of this marker seem to be a specific indication of patients who might be particularly susceptible to antiinflammatory treatment. Similarly, it seems likely that a decrease in MMP-9 may herald an improvement in the health of the ocular surface and patient symptoms.

MMP-9 IN LASIK AND OTHER OCULAR SURGERY

In any ocular surgery, the presence of undiagnosed ocular surface disease can be a hazard. In surgeries such as LASIK or photorefractive keratectomy (PRK), any ocular surface disease can be made worse by the procedure and can result in serious complications. Because dry eye is one of the most common complications of LASIK and PRK, diagnosis and management of ocular surface disease before surgery may lead to less postoperative complications. MMP-9 has been implicated in poor epithelial healing, and epithelial ingrowth after LASIK surgery. Additionally, the reduction in tear function after LASIK may lead to an increase in the concentration of proinflammatory cytokines and MMP-9 in the tear film, which results in dry eyes and insufficient attachment between the corneal flap and the corneal bed. The failure to detect this inflammatory cytokine, which can signal potential complications, might be considered in the future to be below the standard of care.

Not only in LASIK surgery but also in cataract patients and patients undergoing other ocular surgery, corneal ulceration has been reported in patients who have dry eyes and ocular surface disease before the surgery. It is likely that testing for MMP-9 will be the simplest and most reliable way to rule out ocular surface disease and avoid problems such as this. In a practical sense, because of the frequency of ocular surface disease and the unreliability or irreproducibility of many of the tests used to detect ocular surface disease, the detection of elevated levels of MMP-9 might be even more important than the topographical testing for keratoconus and its forme fruste in terms of the frequency of avoiding serious complications.

PRK AND PTK

MMPs play an important role in epithelial regeneration and healing. Coming from the healing epithelial cells or leucocytes that infiltrate, MMPs may cleave Bowman layer, the site of the hemidesmosomes that anchor the epithelial cells. After epithelial closure, MMPs become undetectable. They may well play a role in persistent or recurrent epithelial defects as well as persistent haze, which has been treated with Bowman membrane transplantation; this might be effective for recurrent erosions. Clinical studies indicate that inhibition of MMPs may be as effective as stromal puncture in treating recurrent erosions and preventing epithelial defects either alone (eg, with doxycycline or corticosteroids) or in combination with other therapies.

VERNAL CONJUNCTIVITIS

Just as other ocular surface disease that can damage the surface and the cornea can produce MMP-9, vernal conjunctivitis has been especially correlated with MMP-9 production. It has been suggested that eosinophils and mast cells may be involved in its production, but high levels of MMP-9 have been associated with vernal keratoconjunctivitis. The corneal damage and the ulceration seen in cases of vernal conjunctivitis may be because of the secretion of proteinases and gelatinases, often associated with the eosinophilic basic protein, and a reduction in these mediators may be an early indication of an adequate response to therapy.
OTHER CONDITIONS

Other ocular conditions, once they reach a point of significant surface inflammation, can also be associated with MMP-9 production. These include fungal keratitis, burns, very advanced keratoconus with irregular surface, and other factors that can seriously damage the surface. In these cases, as in others, MMP-9 is a built-in indicator of significant ocular surface disease and also a mediator that can prolong and be responsible for the pathogenesis of the disease. Although these types of ocular conditions may be easily detectable with history or clinical presentation, many more patients have elevated levels of MMP-9 that may cause damage to the ocular surface and may not be detectable without the use of a testing device.

NONOCULAR DISEASE

The measurement of the elevation of MMP-9 has been found to be important in a variety of nonocular diseases. Just as it may be associated with pterygium and basement membrane dissolution, it is apparently involved in joint destruction with rheumatoid arthritis, but more recently, and importantly, it has been found to be a significant factor produced by cancers, especially those that are prone to metastasize. MMP-9 levels have been found to be elevated in breast cancer, aggressive prostate cancer, and bladder cancer. It may play a vital role in cancer metastasis and its measurement may be an important clinical indicator of this. The idea that a simple test could be done on urine, or a fingerstick drop of blood, to gauge the presence and potential metastatic predilection of a tumor, makes this whole field particularly exciting, especially because this testing may now be able to be made available as a simple and practical in-office screening test.

MECHANISMS OF MMP-9 PRODUCTION AND ACTIVITY

A variety of inflammatory mediators seem to have the potential of inducing and being correlated with MMP-9 production. Briefly, the MMP-9 seems to be produced in part through a mitogen-activated protein kinase pathway, but other kinases also seem able to stimulate it. It seems to activate interleukin 1 and is, in fact, correlated with interleukin 1 production. MMP-9 is secreted as a zymogen, a proenzyme, that is physiologically activated by other proteases. Active MMP-9 may be bound and inactivated by tissue inhibitors of matrix metalloproteinases. It is one of a family of MMPs, but for ophthalmology it seems to be the most important.

PRACTICAL MEASUREMENT OF MMP-9 AND ITS AFFECT ON TREATMENT DECISIONS

The earliest work with MMP-9 (gelatinase-B) was done by measuring its ability to dissolve gelatin. This was a relatively complex test that required hours and was difficult to quantitate, but it is still used. It has been improved with a variety of electrophoresis and other techniques (zymography). Western blotting techniques have been used to purify and identify it, as has immunohistochemistry, enzyme leak immunoassays, and MMP-9 capture activity measurements. InflammaDry, a new, inexpensive, disposable single-use assay that provides a result in 10 minutes and can easily be done in the office, changes the utility of MMP-9 from a primarily research phenomenon to one that can and should be used in regular clinical diagnosis as a measure of the health of the ocular surface (Fig. 2). This new assay measures both active and latent MMP-9 (total MMP-9). No direct comparison has been made between elevated MMP-9 levels as determined by the new assay and MMP-9 activity assays that measure enzyme activity. According to the international InflammaDry package insert, clinical study data demonstrate high sensitivity and specificity.

SUMMARY AND CONCLUSIONS

MMP-9 (gelatinase B) is produced in ocular surface disease and is important because it is a reliable indicator of the presence of this disease. In itself, it can cause corneal ulceration and complications after eye surgery such as LASIK, which may make ocular surface disease worse. MMP-9 is elevated in dry eye syndromes and its detection and measurement are not only a reliable indicator of the disease but also may be a reliable indicator of those patients who will respond to antiinflammatory therapy with agents such as cyclosporine or corticosteroids.

The development of an inexpensive and rapid test that can be easily done in the office is vital to the diagnosis of ocular surface disease, and probably the test best correlated with patient symptoms. In fact, elevated levels of MMP-9 may be a more sensitive diagnostic marker than clinical signs. This new test is indicated to detect and avoid complications of ocular surface disease before LASIK and other ocular surgeries as well as to detect hidden cases of dry eye disease that may not be easily identified through the clinical examination, particularly because inflammation is often present long before clinical signs appear. Additionally, the availability of this practical method to detect elevated levels of MMP-9 may facilitate the targeted therapeutic management of symptomatic patients.

REFERENCES


