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Original Publication:

Neil Lagali and Per Fagerholm, Delayed Mustard Gas Keratitis: Clinical Course and In Vivo Confocal Microscopy Findings, 2009, CORNEA, (28), 4, 458-462.
http://dx.doi.org/10.1097/ICO.0b013e31818a7dd0

Clinical Case Report

Delayed mustard gas keratitis: clinical course and in-vivo confocal microscopy findings

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Acknowledgement: This work was supported by the County Council of Östergötland.

ABSTRACT

Purpose: To report the detailed clinical and in-vivo confocal microscopic findings in a patient with delayed-onset mustard gas keratitis observed 20 years after initial exposure.

Methods: Case report. A 38-year-old man who was exposed to mustard gas in Iraq at the age of 19 was examined after presenting with ocular symptoms 17 years after initial recovery from the exposure. Slit-lamp biomicroscopy, corneal topography and in-vivo confocal microscopy was performed on both corneas.

Results: The clinical symptoms were consistent with a delayed-form of mustard gas keratitis, although the patient had clear central corneas and good visual acuity. Confocal microscopic findings included evidence of epithelial abnormalities, necrotic changes in the anterior stroma, subbasal and anterior stromal nerve proliferation, and deep stromal keratocyte activation.

Conclusions: In-vivo confocal microscopy revealed persistent morphologic abnormalities in the anterior stroma of both corneas 20 years after initial exposure to mustard gas. The detection of a population of dendritic cell bodies in the central epithelium and evidence of keratocyte activation and migration in the deep stroma indicated the presence of ongoing sub-clinical processes.

Key Words: cornea, confocal microscopy, mustard gas injury; delayed keratitis

Introduction

Mustard gas has been used widely in chemical warfare, with the vast majority of those exposed to this alkylating agent developing ocular complications.[1,2] Mild and moderate cases of exposure usually resolve after several weeks to months. Severe cases of exposure may resolve or can lead to either chronic inflammation or a delayed-onset keratitis, the latter occurring after a latent asymptomatic period ranging from 1 to 40 years.[1,3-5] While the delayed course occurs in less than 1% of all cases, it can be particularly distressing, with degenerative changes, recurrent and worsening pain and inflammation, ulceration, and progressive loss of vision.[1,3] The pathophysiologic processes underlying this delayed form of keratitis are not well understood. Histopathologic examination of affected corneas after penetrating keratoplasty has revealed abnormal cellular and morphologic features[3,4], however, their origins are unclear. In-vivo confocal microscopy provides a method for gathering detailed structural and morphologic information in the living cornea, and may provide further insights into the mechanisms responsible for the long-term toxicity of mustard gas, particularly in patients where keratoplasty is not indicated. To date, however, only one brief report describing in-vivo confocal microscopy findings in a patient with delayed-onset keratitis could be found.[5] Herein we report detailed clinical and in-vivo confocal microscopic observations of persistent changes in two corneas from a patient with delayed-onset mustard gas keratitis, observed 20 years after exposure.

Case Report

The patient was a Kurdish civilian exposed to mustard gas in Iraq in 1988 during the Iran – Iraq war. Immediately following exposure he suffered acute damage to both eyes resulting in blindness lasting a period of six months. Thereafter the patient's vision returned and he became

asymptomatic a year after the injury and remained so until Sept 2006. He then presented with pain, soreness and a burning sensation in his eyes, along with excessive watering and severe photophobia. Best-corrected visual acuity was 20/20 in both eyes. The right cornea was transparent; however, a mild conjunctival irritation was noted. The left cornea was transparent centrally, while a crescent-shaped peripheral scar was noted from 5 to 9 o'clock on the nasal inferior side, extending 1-2mm over the limbal region into the sclera. Rose Bengal stain was applied to both eyes, with no corneal staining observed. A light limbal staining, however, was observed in the right eye, while an 8mm-diameter conjunctival region 4-5mm away from the limbus (nasally) stained positive in the left eye. The inner side of the left palpebral conjunctiva also stained positive. The lid margins were slightly irregular. No symblepharon or blepharitis was noted, and the lens and fundi were normal. The patient had been treated with 2% cyclosporine (ex tempore) without effect and a lubricant was applied frequently (sodium hyaluronate 0.1 %, HyloComod, Ursapharm, Artzmittel, Saarbrücken, Germany). Due to continuing symptoms, he was referred to our clinic.

In May 2007, the patient was experiencing dry eye symptoms in the left eye, and was unsatisfied with the effect of the medication. Schirmer's test revealed normal tear production (15mm in 5 min.), while a reduced tear break-up time was noted (6s/10s, OD/OS) and the quality of the tear film was poor. Three drops of a topical steroid-antibiotic mixture (oxytetracycline hydrochloride 0.5 %, hydrocortisone acetate 1.5 % and 10 000 IE/ml polymyxinB sulphate, Terracortil with polymyxin B, Pfizer) was administered to the left eye, and 3 to 6 drops of a tear substitute (hypromellos 0.5%, Isopto Plain, Alcon) was applied to both eyes. In September 2007 the patient returned with an increased burning sensation and irritation in the left eye. Schirmer's test and tear break-up time remained unchanged and intraocular pressure was normal (14mm Hg

in both eyes). Punctal plugs were inserted (into both lower puncta), the lubricant was changed to povolon 50 mg/ ml (Oculac, 50mg/ml, Novartis) 5 times per day, and Terracortril with Polymyxin eye drops 3 times per day was maintained in the left eye. At follow-up visits in December 2007 and February 2008 the dry-eye related symptoms had subsided, however, in June 2008, Schirmer's test indicated moderate wetting in the right eye (9mm/19mm, OD/OS) and a reduced tear break-up time (4s/5s, OD/OS). Cochet-Bonnet esthesiometry (Luneau, France) indicated a sensitivity of 60mm centrally, nasally and temporally in the right eye and 60mm centrally and 50mm nasally and temporally in the left eye.

Methods

Clinical slit-lamp photographs were taken and corneal topography (Orbscan II ,Bausch & Lomb) was measured. In-vivo confocal microscopy examination of both eyes was performed in December 2007 and in February 2008, using a laser scanning system (HRT III-RCM; Heidelberg Retina Tomograph III Rostock Corneal Module, Heidelberg Engineering GmbH, Heidelberg, Germany), fitted with a 63× water immersion microscope objective with a numerical aperture of 0.95 (Zeiss, Jena, Germany). Prior to examination, the patient received a drop of topical anaesthetic (0.4% oxybuprocaine hydrochloride, Chauvin Pharmaceuticals, Surrey, England) in each eye, and tear-gel (2mg/g carbomer, Novartis, Täby, Sweden) was used for optical coupling between the cornea and the microscope objective. Several scans through the full corneal thickness were taken in both eyes.

Results

Slit lamp biomicroscopy revealed a clear central cornea bilaterally and a crescent-shaped peripheral scar in the left cornea (Fig 1A). Mildly tortuous conjunctival vessels were observed (Fig 1B) and the lid margins were somewhat uneven (Fig 1C). Topography revealed a central thickness that was likely normal while inferior corneal thickness was reduced by about 200μm from lateral and superior regions in both eyes. In-vivo confocal microscopy revealed similar features in both eyes. In the central cornea, the superficial epithelium could not be adequately visualized due to a high interface reflection, however, epithelial wing cell layers contained reflective cellular inclusions (Fig 2A) while the basal epithelium was populated with dendritic cell bodies lacking dendrites (Fig 2B; density in frame: 210 cells/mm^2). A Bowman's layer fold was noted in the right eye at the level of the basal epithelium (Fig 2C). Long, prominent, parallel-running subbasal nerves were noted just above Bowman's membrane (Fig 2D,E) with an abnormally high incidence of nerve branching. A highly irregular network of thicker nerves was observed just posterior to Bowman's membrane in the most anterior stroma. This nerve network consisted of unusual circular node-like structures from which multiple nerve fibers emanated (Fig 2F), and in one region the nodes were connected in a chain-like formation (Fig 2G). Thicker anterior stromal nerve trunks gave rise to the nerve network layer (Fig 2H), and in some cases the trunks extended up to Bowman's layer (Fig 2I). The anterior stroma was notably devoid of normal-appearing keratocyte nuclei; instead, opaque bodies of various size and form populated the anterior stroma, and were interspersed with punctuate microdots and spindle-like structures (Fig 2J,K). In the mid-stroma, spindle-like structures were observed along with a few keratocyte nuclei and stromal nerves (Fig 2L). The posterior stroma was more densely populated with keratocytes, with highly reflective keratocyte nuclei co-located with reflective stromal

inhomogeneities (Fig 2M) or elongated in nature (Fig 2N). In both corneas, the endothelium appeared normal.

Discussion

This patient exhibited typical symptoms of delayed-form mustard gas keratitis, including excessive burning, tearing, pain, photophobia, and moderate to severe dry eye[3,6]. The 17-year latency period for delayed symptoms is slightly longer than the 1 – 15 yr period reported in a review of 17 cases of delayed-form mustard gas keratitis in victims of the Iran – Iraq war[3], but is typical for delayed mustard gas complications seen in a group of patients gassed during World War I.[7] The clinical abnormalities observed in our case included stromal scarring, a mild vascular tortuosity in the conjunctiva, corneal thinning, and a reduced tear break up time – findings which have been previously reported in other delayed-form cases.[3,6] Both the corneal thinning and the peripheral scar observed in our patient were located inferiorly, which is consistent with protection of the upper cornea by the eyelid.[1] An asymmetry, indicated by the scarring and significant conjunctival staining in the left eye, is also a common finding.[3] Furthermore, the patient reported reduced ocular discomfort in colder climates, a finding also previously noted.[3] Notably, however, several features noted in our patient are less commonly reported: good bilateral visual acuity, an absence of corneal opacities or ulceration, and absence of corneal vascularization. Although the initial mustard gas exposure and the immediate ocular effects were severe, the patient's visual acuity has been spared in the longer-term, and the corneas appear to be stable without degenerative changes.

By confocal microscopy, dendritic cell bodies lacking dendrites were seen interspersed among subbasal nerves at the basal epithelium. The cell bodies were observed in both central and

peripheral regions, with the relatively dense central corneal population suggestive of inflammatory activity.[8] Additionally, circular structures with increased reflectivity at the basal epithelium and the more superficial inclusions at the wing cell layers were atypical. Epithelial irregularities have been noted in a number of delayed-onset cases[3,5,9], and histopathologic studies of excised corneal buttons from patients who underwent keratoplasty revealed a thinned or absent epithelium and Bowman's layer.[3,4] Experiments in rabbits have revealed an initial destruction of the epithelium immediately following mustard exposure, followed by abnormal epithelial regeneration indicative of a pathological healing process.[10,11] Immunohistochemical studies have suggested that disruption of the normal epithelial repair process may have resulted from mustard-induced conformational changes in basement membrane proteins.[10] Immunological reaction to these structurally modified proteins has been proposed as a model for the pathogenesis of the delayed-form of mustard gas keratitis.[1,3] Our observation of a population of dendritic cells and unidentified cell-like inclusions in the central cornea (resembling globular cells seen in an inflamed cornea[8]) may lend support to this theory. Javadi et al.[3] noted that in delayed-onset cases, unpredictable, recurrent episodes of inflammation occur, and in our case delayed symptoms accompanied by inflammation may have resulted in the presence of dendritic cells observed in the central epithelium.

Notably, we observed several interesting features in our case relating to corneal nerves. While the regeneration of subbasal nerves after chemical injury has not previously been reported, subbasal nerves are known to regenerate very slowly after penetrating keratoplasty[12] and several studies have shown only sparse subbasal nerve regeneration decades later.[13-15] Following refractive surgery, subbasal nerves have returned to normal densities only after several years.[16] In the present case, the presence of an intact subbasal nerve plexus indicates the factors resulting

in persistent epithelial and stromal changes did not preclude long-term nerve regeneration or proliferation. Although in our case it is unclear whether corneal subbasal nerves have taken months, years, or decades to return, their presence in significant numbers is important, as they are the source of epithelial nerves and are essential for a healthy ocular surface.[17] As a high mechanical touch sensitivity was measured in both eyes, it can be assumed that the subbasal nerves have populated the more superficial epithelial cell layers and are functional. Immediately below Bowman's layer, a network of nerves with irregular circular node-like structures appeared to originate from thick nerve trunks not usually observed in the anterior stroma.[18] The dense distribution of nerves and substantial branching observed anterior and posterior to Bowman's membrane are evidence of abnormally accelerated nerve growth and proliferation. Whether the observed nerve proliferation was related to a pathological epithelium or stroma is unknown; however, given the close functional relation between corneal nerves, stromal cells and the epithelium,[17] an association is plausible.

The abundance of nerves found in our case and the corresponding high touch sensitivity in both corneas appears to conflict with a long-term loss of corneal sensitivity reported in mustard gas casualties.[3,11]. In general, the effect of mustard gas on corneal nerve presence and morphology has not previously been studied in detail, and further research is required to describe the course of neurogenesis following chemical exposure and its clinical implications.

Another prominent stromal finding in our case was a complete lack of keratocyte nuclei in the anterior stroma. In a normal cornea the anterior stroma usually contains the greatest concentration of keratocytes.[15,19] In several studies of animal and human corneas exposed to mustard gas, stromal irregularities have been noted and included the presence of edematous regions, sediment deposits, scar tissue, necrotic keratocytes and inflammatory cells.[4,5,9-11] Our

findings of Bowman's membrane folds, stromal deposits, spindle-like structures, punctuate microdots and an absence of keratocytes in the anterior stroma indicate pathological basal epithelial and anterior stromal changes that persisted in the long term, echoing features reported in the aforementioned studies. In particular, the spindle-like structures observed may represent necrotic keratocytes, also seen in histologic sections[4] and confocal images[5] and believed to play a role in the pathogenesis of delayed-form keratitis. The long-term persistence of anterior stromal irregularities may be a consequence of the reaction of stromal collagen with mustard gas, which has been shown to produce a more rigid, less soluble, and more enzyme-resistant form of corneal collagen in vitro.[20]

Morphologically, the anterior stroma consisted of necrotic-like tissue with localized regions of high reflectivity within a transparent (dark) background, which distinctly differs from the organization of reflective fibroblast cell bodies in a mesh-like network, characteristic of typical scar tissue.[21] Anterior corneal nerves, not usually visible within fibrotic scar tissue, were present in our patient. Unlike surgical trauma, after which keratocytes quickly repopulate the stroma following an initial apoptotic phase,[22] initial keratocyte apoptosis and/or necrosis after chemical exposure in our patient was not accompanied by repopulation, even after 20 years. From the observed morphology of the anterior stroma we postulate that a modified form of stromal collagen appears to have impeded normal wound-healing processes. The presence of keratocytes (both normal-appearing and activated) in the mid- and posterior stroma suggests modification localized to the anterior third of the stroma. Among the keratocytes observed within the posterior stroma, nuclei often appeared highly reflective with translucent cell bodies visible, suggesting an activated state. The additional presence of elongated, migratory-type keratocyte nuclei suggests that the cornea may still be in a state of flux. It has been noted that

delayed-form cases often exhibit unpredictable exacerbations and remissions in their clinical course;[3] our findings of dendritic cells and activated keratocyte nuclei at the microscopic level indicate ongoing activity underlying the macroscopic clinical observations.

Finally, while limbal stem cell deficiency has been noted following mustard gas exposure,[3] in the present case the epithelium in both corneas remained intact without lesions. As epithelial integrity, transparency, and gross morphology in our case appeared normal and no signs of vascularization or conjunctivalization were observed, a partial limbal stem cell deficiency was unlikely to have been present. [4]

In conclusion, in-vivo confocal microscopy in a patient with delayed-form mustard gas keratitis revealed persistent changes to the corneal epithelium, stroma, and nerves 20 years after the initial exposure. Morphological evidence of necrotic stromal changes, stromal modification, and inflammatory processes indicate factors that may contribute to the infliction of long-term corneal damage. Further confocal microscopic investigations in a larger group of patients may be useful in elucidating the specific mechanisms underlying the clinical manifestations of delayed-form mustard gas keratitis.

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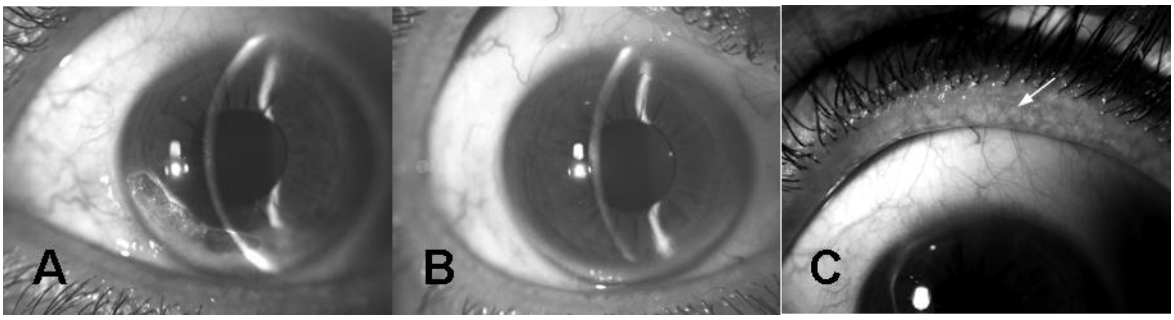

Figure 1. Slit-lamp biomicroscopy 20 years after mustard gas exposure. A, clear central cornea and peripheral scar in the left eye. Note the mildly tortuous conjunctival vessels. B, clear cornea in the right eye. C, unevenness in the eyelid margin (arrow).

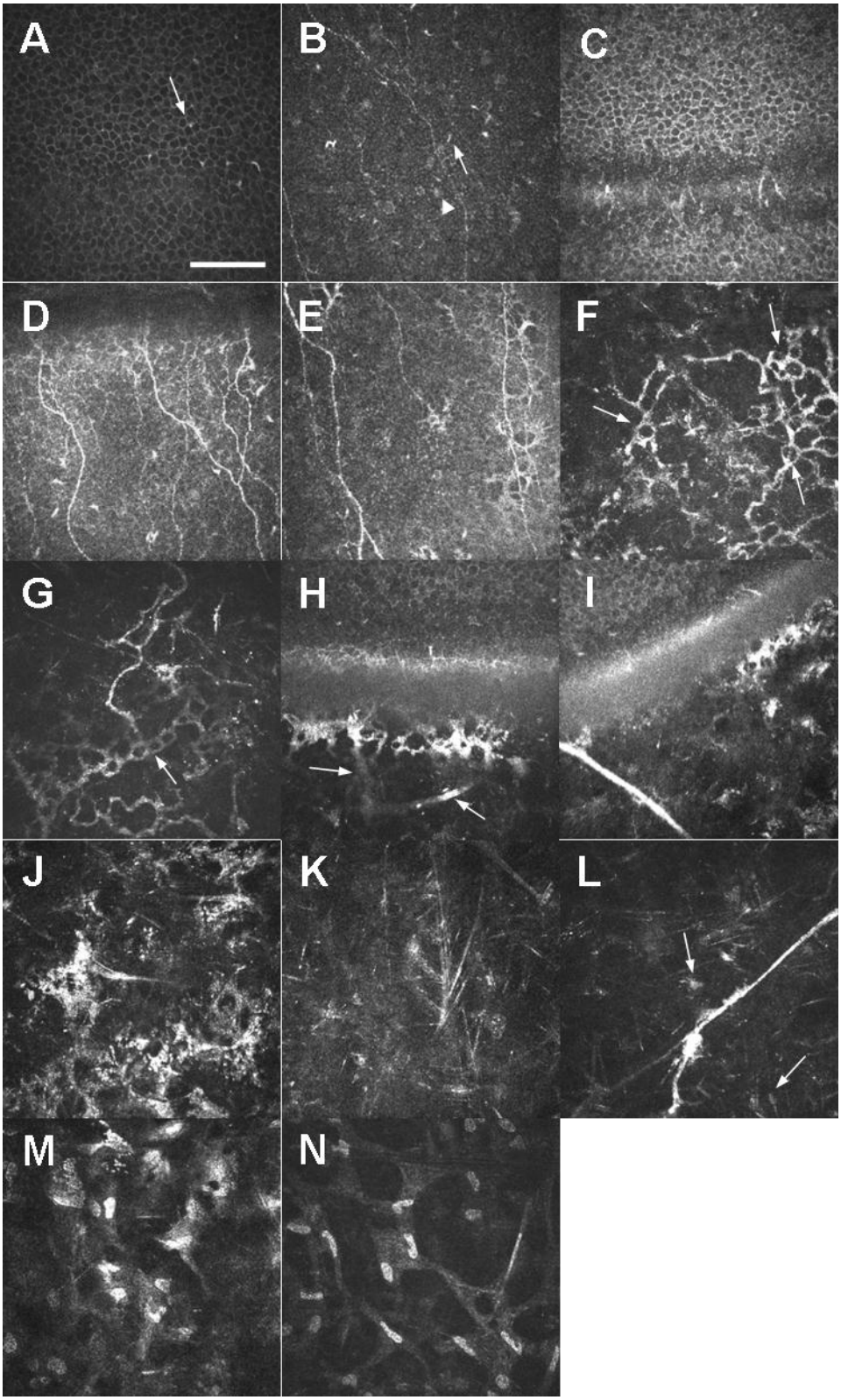
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Figure 2. In-vivo confocal microscopic appearance of centrally clear corneas 20 years after mustard gas exposure. All images are 400 × 400μm (scale bar 100 μm) and depth values are referenced to the corneal surface. A, OD: epithelial wing cells of the central cornea with reflective cell-like inclusions (arrow); depth 36μm,. B, OD: basal epithelial cells of the central cornea, with numerous dendritic cell bodies lacking dendrites (arrow), and reflective circular structures (arrowhead, diameter 8-10μm); depth 49μm. C, OD: upward fold of Bowman's layer penetrating the basal epithelium. Note the presence of subbasal nerves and dendritic cell bodies anterior to Bowman's layer; depth 47μm. D, OD: subbasal nerves with multiple fine branches, and dendritic cell bodies interspersed through this layer; depth 68μm. E, OD: multiple branching nerves in the anterior stroma crossing Bowman's layer and forming a subbasal nerve plexus. F, OD: irregular nerve network with node-like nerve structures (arrows) just below Bowman's layer; depth 96μm. G, OD: abnormally tortuous nerves of varying diameter in the anterior stroma with a chain-like nerve configuration (arrow); depth 110μm. H, OD: oblique section indicating thick anterior stromal nerve trunks (arrows) branching to form a nerve network below Bowman's layer. I, OS: thick stromal nerve trunk extending anteriorly to Bowman's layer. J, OD: anterior stromal opacities, punctuate microdots (diameter 1-4μm) and spindle-like structures; depth 112μm. K, OS: spindle-like structures and microdots in the anterior stroma; depth 120μm. L, OD: mid-stroma with a nerve, spindle-like structures, and a few keratocyte nuclei (arrows); depth 214μm. M, OD: reflective posterior stromal keratocyte nuclei with cellular bodies partially visible; depth 443μm. N, OD: elongated posterior stromal keratocyte nuclei with cell bodies visible; depth 465μm.