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BRIEF REPORT

Successful Treatment of *Mycobacterium chelonae* Keratitis Within a Corneal Transplant Using Intrastromal Amikacin Injections—A Case Report Demonstrating the Fundamental Principles and Challenges of Infective Keratitis Management and Novel Therapeutic Approaches

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Mycobacterium chelonae keratitis is rare and difficult to treat. This is the first known case worldwide of effective treatment using intrastromal amikacin injections in a corneal transplant recipient who had metastatic breast cancer. The challenges and principles of management, applicable to other causes of infective keratitis, are reviewed.

Keywords. corneal transplant; infective keratitis; intrastromal amikacin injections; *Mycobacterium chelonae*; nontuberculous mycobacteria; penetrating keratoplasty.

Mycobacterium chelonae keratitis is due to a ubiquitous, fast-growing nontuberculous mycobacteria (NTM) found in water and soil, causing visual loss and morbidity [1–3]. Risk factors include ocular trauma, procedures, and foreign bodies; ocular topical steroid use; host immunocompromise; and chronic ocular surface disease [2–5]. Symptoms can include ocular pain, photophobia, and reduced vision; signs can be insidious or mimic more common pathogens; and delayed microbiological diagnosis is common [2–3].

M. chelonae keratitis is rare but accounts for half of all NTM keratitis. Worldwide, there have been fewer than 300 cases of *M. chelonae keratitis* ever reported, including around 10 cases complicating penetrating keratoplasty [2–6].

Current literature recommends management via topical steroid cessation, prolonged treatment with combination topical antibiotics (quinolone, aminoglycoside, and macrolide), and supportive care. Systemic antibiotics (macrolide and tetracycline) are second-line adjuncts. Surgery (debridement, corneal transplant, or enucleation) is reserved for recalcitrant cases. Relapse rates are high, and long-term visual impairment occurs in 20%–50% of cases [2–3, 5].

In practice, treating *M. chelonae* keratitis is challenging due to corneal anatomy, physiology, and pharmacology; delayed identification and directed therapy, limitations of antimicrobial susceptibility testing; poor tolerability of antimicrobial therapies; and paucity of evidence for their use [2, 3, 7].

Infection may occur in 1 or more of the 5 corneal layers (Figure 1A). In a normal cornea, the layers are avascular, have variable intercellular junctions, and have alternating lipophilic and hydrophilic properties. The pharmacokinetics of antimicrobial tissue penetration via diffusion to the affected corneal layer(s) is therefore complex and poses a major challenge to effectively managing corneal infections [7–9].

There are no standardized clinical breakpoints for topical therapy of mycobacterial infections. Availability of ophthalmic preparations (topical eyedrops/ointment or intrastromal/intravitreal injections) is limited, with little evidence as to the formulation, dose frequency, tissue penetration, or route of administration [9]. Barriers to adherence include ocular pain and high-frequency dosing, sustained over weeks to months. Systemic antibiotics may be added; however, their corneal penetration is unknown [2, 3, 8, 9].

CASE

A 38-year-old female developed keratitis in corneal transplant tissue after emergency penetrating keratoplasty (PK), performed for left eye corneal perforation complicating chronic keratoconjunctivitis sicca (KS). This was in the setting of metastatic human epidermal growth factor receptor 2–positive ductal carcinoma of the breast, first diagnosed 10 years prior.

KS was multifactorial and largely irreversible due to (1) mucous membrane dryness associated with the patient's palliative emtansine-traztuzumab oncology regimen; (2) tear film deficiencies due to cerebral metastases affecting trigeminal, abducens, and facial nerves, which collectively impaired lacrimation, blink reflex, eye movement, and voluntary eyelid closure; and (3) neurovascular toxicity from whole-brain irradiation [10]. To minimize the risk of KS recurrence in the PK graft, emtansine was changed to carboplatin at the time of PK, and permanent

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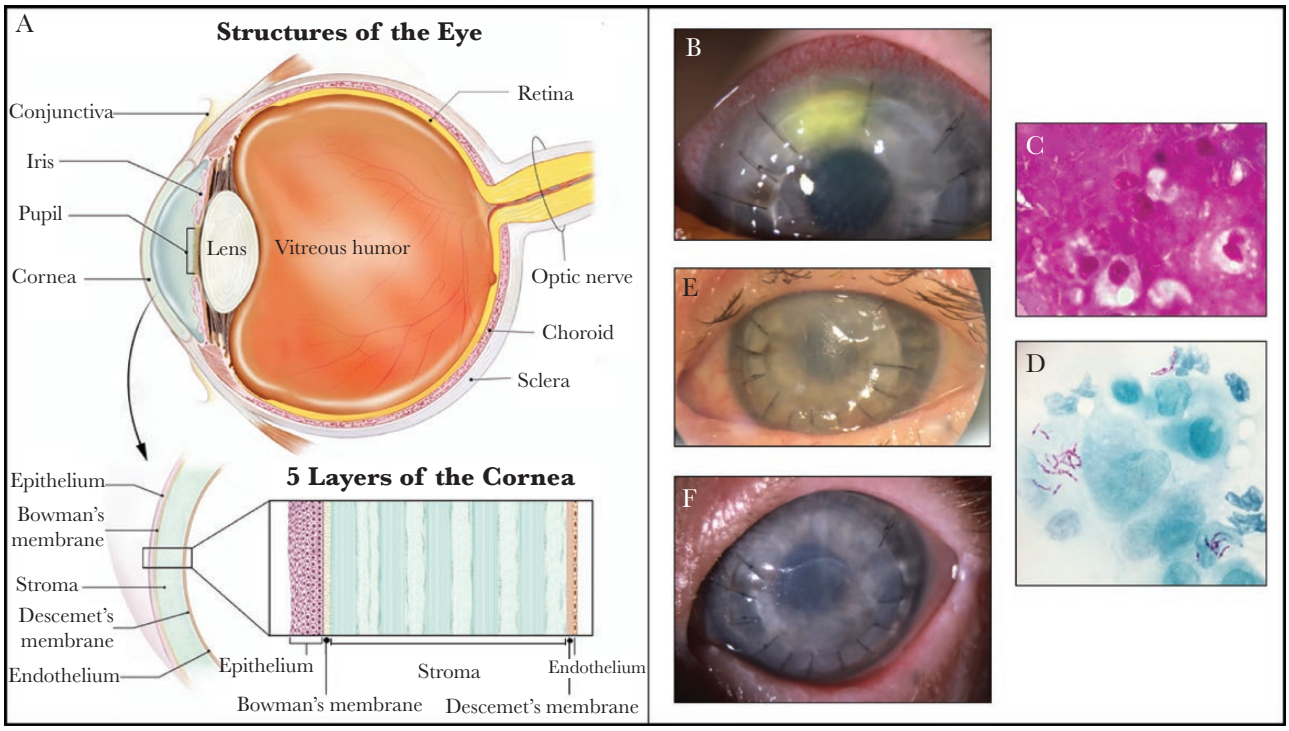

Figure 1. A, Diagram of the eye demonstrating corneal layers and relation to other structures, reproduced with permission from the National Eye Institute, United States of America. B, Left eye epithelial and stromal infiltrates within penetrating keratoplasty (PK) graft at time of corneal sampling. C, Gram stain from corneal sample showing branched rods that do not take up the stain. D, Ziehl-Neelsen stain from corneal sample showing beaded acid-fast bacilli. E, One week after completing the course of intrastromal amikacin injections: infiltrates resolved, but corneal haze and signs of PK graft failure. F, Eight weeks after completing the course of intrastromal amikacin injections: retained PK corneal graft has vascularized due to steroid cessation.

tarsorrhaphy was performed 5 weeks later to improve eyelid closure.

The corneal PK donor tissue was retrieved 17 hours after donor death and preserved normothermically (34°C, organ culture media [OCM], Eagle's Minimum Essential Media, 2% fetal bovine serum with penicillin 100 U/mL, streptomycin 100 µg/mL, amphotericin B 0.25 µg/mL) for 12 days. On day 12, it was transferred to the final thinning media (OCM with 5% Dextran 500) and transplanted the following day. Microbiologic monitoring was conducted throughout the process, with no growth of any microorganisms detected.

Standard PK postoperative topical agents were prescribed: 4 times daily prednisolone minims (single dose vials), chloramphenicol ointment, lubricating drops, and vitamin A ointment. Preparations were preservative-free where possible to minimize corneal inflammation, and topical dispensers were replaced every 28 days. The bandage contact lens was removed per routine practice at 4 weeks post-PK.

No acute postoperative issues were encountered. An infiltrate at the graft-host junction, first noted 4 weeks post-PK, was managed for possible rejection by increasing steroids and had regressed by week 6. At week 12, a hazy opacity was observed and empiric ofloxacin eyedrops (1% 2-hourly) commenced. At week 14, there was no corneal defect. At week 15, 3 deep stromal infiltrates were evident with overlying epithelial defects and 2+ anterior chamber cells but no hypopyon (Figure 1B). The patient reported no symptoms of pain, photophobia, acute visual changes, or changes to her baseline dry eye symptoms.

METHODS

Corneal scrapings of the stromal infiltrate were collected at week 15 and processed for microscopy; bacterial, mycobacterial, and fungal culture; and polymerase chain reaction (PCR) for herpes simplex and varicella zoster viruses. Westmead Reference Laboratory performed PCR for isolate identification and performed microbroth dilution for mycobacterial susceptibility testing.

RESULTS

Microscopy demonstrated >30 leukocytes per low power field (10× magnification) and branched rods that did not take up Gram stain (Figure 1C). Beaded acid-fast bacilli were detected on Ziehl-Neelsen stain (Figure 1D). White spreading colonies were detected on blood agar at day 4. MALDI-TOF failed to identify the isolate using standard and formic acid extraction methods. *M. chelonae* was identified by PCR. In vitro susceptibility testing reported susceptibility to amikacin (minimum

inhibitory concentration [MIC] 8), clarithromycin (MIC <0.006), and linezolid (MIC 2); intermediate susceptibility to tobramycin (MIC 4); and resistance to ciprofloxacin (MIC >4), moxifloxacin (MIC 8), cefoxitin (MIC 128), imipenem (MIC >64), trimethoprim-sulfamethoxazole (MIC 4/76), and doxycycline (MIC >16).

Topical steroids were ceased when *M. chelonae* was identified; however, keratitis worsened on the following empiric topical agents, which overall were poorly tolerated. Aminoglycoside eyedrops (gentamicin 0.9% hourly for 1 week, replaced by amikacin 2.5% hourly) were associated with pain and drop fatigue. Azithromycin eyedrops (1% twice daily, used because no topical clarithromycin was available) were ceased after 4 days due to pain. Topical lignocaine, to optimize adherence, was avoided due to its risks of delayed presentation for worsening epitheliopathy and worsening infection. Ofloxacin eyedrops (1% hourly), although well tolerated aside from drop fatigue, were ceased due to isolate resistance. No topical preparation of linezolid was available.

Systemic antibiotics were avoided initially due to negligible tissue penetration and risks of interactions with her oncology regimen. Oral azithromycin 250 mg daily had been started pending availability of azithromycin eyedrops, but was poorly tolerated and ceased after 2 weeks.

Intrastromal antibiotic injections were avoided initially due to concerns of seeding deeper infection. However, hypopyon developed after 2 weeks of topical directed therapy, suggestive of progressive disease.

Intrastromal amikacin injections (0.1 mL of 2.5 mg/mL) were commenced 2 weeks after corneal scrapings, with 11 doses in total being administrated over 7 weeks, while continuing amikacin eyedrops. Amikacin injections were associated with rapid clinical improvement (Figure 1E) and were well tolerated, as corneal graft tissue is insensate—in contrast to painful eyedrops, which diffused over her sensate native sclera and conjunctiva. Amikacin eyedrops ceased 10 days after the final injection due to resolution of infiltrate. No local or systemic adverse events were identified during or after the course of intrastromal amikacin injections.

To prevent superinfection of the healing epithelial defect, topical tobramycin (eyedrops 0.3% 6 times daily, later changed to ointment 0.3% 4 times daily) was instituted after the third intrastromal amikacin injection and continued until the defect healed 3 months later.

Outcomes were acceptable (Figure 1F). Primary aims were achieved: clinical resolution of PK epithelial and stromal infiltrates, corneal stabilization, graft retention as a physical barrier to prevent invasive polymicrobial ocular infection, and avoidance of further surgery per strong patient preference. However, as anticipated, cessation of topical steroids resulted in vascularization of the transplanted cornea and consequent complete loss of ipsilateral visual acuity (VA). Her best VA post-PK had been poor (6/36), with vision further impaired by diplopia from her abducens nerve palsy. Right eye VA remained intact.

At subsequent follow-up over 4 months, until her death from progressive metastatic malignancy, there was no clinical evidence of *M. chelonae* PK keratitis relapse.

DISCUSSION

We hypothesize that intrastromal amikacin delivery maintained a high concentration to achieve sterilization and rapid clinical improvement, whereas topical amikacin had insufficient stromal penetration. The role of prophylactic topical tobramycinin the sustained resolution of keratitis is uncertain, given its intermediate susceptibility, unknown stromal penetration, and that clinical improvement had preceded its use. Overall, we deduce that resolution of infection was most likely due to intrastromal amikacin injections.

The pharmacological limitations of treating ocular NTM infections (and other sanctuary sites) are not covered by general NTM treatment guidelines [11]. Our decision to use intrastromal amikacin was extrapolated from evidence for intravitreal therapy in endophthalmitis [12]. We used monotherapy, against the guidelines, due to the unavailability and/or intolerability of alternatives. Ocular formulations of clarithromycin and linezolid were unavailable and had virtually no supporting evidence [13].

This report demonstrates key therapeutic concepts that may be cautiously applied to other cases of infective keratitis. Intrastromal antimicrobial injections could benefit eligible patients such as those with refractory stromal infections, isolates that are multiresistant or susceptible to antimicrobials with unfavorable corneal penetration, strong preference to avoid ocular surgery, and for whom the benefits of intrastromal injections may outweigh risks. Substantially lower frequency of intrastromal injections, compared with the burden of eyedrop fatigue and pain for native and PK keratitis patients, may improve compliance and thereby enhance clinical outcomes. Pain or fear of injection may preclude some patients with sensate native corneas compared with insensate transplanted corneas.

Intrastromal antimicrobial injections could be considered for induction and/or suppressive treatment for NTM keratitis, as guided by clinical response and tolerability. Optimal duration and frequency of either phase are unknown and must balance relative risks of iatrogenic complications and antimicrobial resistance. Other factors supporting extended suppressive therapy may include host immunocompromise and/or presence of retained prosthetic material with biofilm potential.

Topical steroid cessation for PK infective keratitis is challenging, and, although recommended in current NTM keratitis guidelines, this decision ultimately rests with the ophthalmologist. Restarting topical steroids in order to minimize corneal vascularization and inflammation should be approached cautiously, as topical steroids may mask signs of deteriorating

infection. Systemic steroids additionally pose multisystem adverse consequences of their chronic use. Prudent topical steroid reintroduction after effective intrastromal antimicrobial injections could be supplemented by serial corneal sampling to assess for microbiological cure or persistence.

Surgical management of NTM keratitis may be more appropriate in most cases of recalcitrant infective keratitis for patients with an active, rather than palliative, focus of care.

Finally, this report highlights the need for microbiological and pharmacological research to further define ocular site-specific break points and formulations for alternative routes of local administration. Avoiding systemic routes has merit to minimize toxicity and drug interactions—especially because the normally avascular cornea is deprived of hematogenous drug delivery, and antimicrobial levels in lacrimal secretions are uncertain. Emerging antimicrobial resistance commands the ongoing need for novel drug development.

The supplying eye bank and the Therapeutic Goods Administration were notified of the adverse outcome associated with human transplant. This is the first known case of *M. chelonae* PK keratitis in >35 000 Australian keratoplasties in the last 35 years [14]. The absence of any microbiological contamination being detected during the preservation and transfer phases of the cornea makes it extremely unlikely that the donor tissue was the source of this rare and previously unrecorded (in Australia) etiology of PK keratitis.

CONCLUSIONS

To our knowledge, this is the first case report worldwide to demonstrate that intrastromal amikacin injections can be effective, safe, and well tolerated in patients with PK graft *M. chelonae* keratitis and may be of use in other etiologies of infective keratitis. Challenges of corneal therapy include the availability and tolerability of ophthalmic antimicrobial preparations, tissue penetration, and absence of standardized susceptibility testing. Collaboration between clinicians, microbiologists, and subspecialty pharmacists in infectious diseases and ophthalmology is crucial to facilitate appropriate regimens.

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