Cornea 20(7): 715–719, 2001.
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Intracameral Amphotericin B

Initial Experience in Severe Keratomycosis

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Purpose. Fungal keratitis is a significant cause of ocular morbidity in India. The most commonly implicated fungi are *Aspergillus* spp. Patients often present with hypopyon, which usually contains fungal elements. The treatment is difficult owing to poor intraocular penetration of most available antifungal agents. This study evaluated the results of intracameral injection of amphotericin B in natamycin resistant cases of severe keratomycosis. **Methods.** Three patients of culture proven *Aspergillus flavus* corneal ulcer with hypopyon not responding to topical natamycin 5%, amphotericin B 0.15%, and oral itraconazole were administered intracameral amphotericin B. The first case received 7.5 μg in 0.1 mL followed by two subsequent injections of 10 μg in 0.1 mL each, the second case received two injections of 10 μg in 0.1 mL, and the third patient received a single dose of 10 μg in 0.1 mL. Culture of the aqueous sample also grew *A. flavus* in all three cases. **Results.** All three cases responded favorably, with the ulcer and hypopyon clearing completely. There was no clinical evidence of corneal or lenticular toxicity in any patient. **Conclusions.** Intracameral amphotericin B may be a useful modality in the treatment of severe keratomycosis not responding to topical natamycin. It ensures adequate drug delivery into the anterior chamber and may be especially useful to avoid surgical intervention in the acute stage of the disease.

Key Words: Intracameral—Amphotericin B—Antifungals—Keratomycosis.

Fungal keratitis is a significant cause of ocular morbidity in people engaged in agriculture. The entity is often underdiagnosed and inappropriately treated. In tropical climes, filamentous fungi are the most common etiologic agents,[1–4] with *Aspergillus* spp accounting for the majority in India.[3,4] Sometimes the hyphae may orient themselves perpendicular to the corneal lamellae,[5] conferring on them the ability to penetrate an intact Descemet's membrane and colonize the anterior chamber. The hypopyon in these cases of deep keratomycosis usually contains fungal elements (unlike that in bacterial ulcers with perforation where the hypopyon is sterile).[6] Fungal hypopyon is a particularly difficult entity to treat because corneal penetration of most topically applied drugs is poor, improving slightly with scraping of the epithelium.[7]

The selection of appropriate therapy for fungal infection remains an unsettled question. Although many antifungal agents are available, treatment with these agents is complicated by a narrow spectrum of activity, lack of effective penetration into the eye, and toxicity.[8] Current strategies for the treatment of fungal corneal ulcers rely on locally administered antifungal agents, usually by the topical route. Although some antifungal agents can be given by subconjunctival injection, toxicity is a major problem, particularly with the polyenes.[9]

Natamycin 5% is considered the drug of choice for filamentous fungi.[6,8,9] However, resistance to natamycin and its inability to treat deep stromal infection and hypopyon are causes for concern, making treatment all the more difficult. Fluconazole 0.3% was recently introduced as an ophthalmic preparation, but its spectrum of activity is primarily against yeasts.[10]

The efficacy of amphotericin B against a variety of fungal pathogens is well documented.[8–10] It has been tried as a topical antifungal agent in low concentrations[11] and is widely used intravitreally in culture-proven fungal endophthalmitis. However, its potential as an intracameral agent has not been exploited clinically, although the few reports available are encouraging.[12–14]

Presented here are three cases of culture-proven *Aspergillus flavus* corneal ulcer with hypopyon, not responsive to systemic and topical antifungal agents, which were treated with intracameral amphotericin B. The corneal ulcer and hypopyon in all three cases responded favorably.

CASE REPORTS

Case 1

A 35-year-old man presented with pain, redness, and diminution of vision in the left eye after injury caused by a wheat stalk 1 month previously. He was diagnosed with a left corneal ulcer by a local ophthalmologist and started on 0.3% topical ciprofloxacin, 0.3% fluconazole, and 1% atropine. There was no improvement in the ocular status for 3 weeks, after which he was referred to our institution. He presented to our cornea service 1 month after the injury.

On presentation, the vision in the left eye was counting fingers with accurate light projection. There was a large 8.0-mm × 6.0-mm epithelial defect and infiltration with hyphate margins involving

Submitted November 14, 2000. Revision received May 1, 2001. Accepted May 3, 2001.

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50% to 60% of the stroma. There was an associated hypopyon measuring 1.0 mm, which was nonmobile on change in position. No fundus details were visible. B-scan ultrasonography (US) revealed a normal posterior segment. The right eye was normal.

A corneal scraping revealed hyphae on 10% potassium hydroxide (KOH) smear. He was started on 5% topical natamycin every hour, 0.3% ofloxacin every 4 hours, 1% atropine three times daily, and 0.5% timolol drops twice daily. One week of this therapy resulted in no appreciable response.

The hypopyon increased and the infiltrate started to involve the deeper stroma. Topical amphotericin B 0.15% every half hour, along with 200 mg of systemic itraconazole twice daily, was added to the treatment at this stage. The infiltrate responded to this treatment and cleared from the anterior stroma, leaving a posterior stromal abscess.

The fungal culture from the corneal scraping revealed *A. flavus*. The hypopyon increased further at this stage to involve nearly half the anterior chamber and measured 6.0 mm in height (Fig. 1A). One month after institution of systemic itraconazole and topical amphotericin B, an aqueous tap was performed, and 7.5 μg of amphotericin B in 0.1 mL was injected into the anterior chamber. The aqueous fluid revealed hyphae on KOH wet mount examination. The hypopyon decreased by 2.0 mm 48 hours later, but the posterior corneal plaque persisted. Intracameral amphotericin B 10 μg was repeated 72 hours after the first injection. The patient responded well, and the hypopyon as well as the posterior corneal plaque started to decrease. It remained static after 4 days, when a final 10 μg intracameral amphotericin B was injected.

Culture of the first aqueous sample grew *A. flavus*. The ulcer and the hypopyon completely resolved 3 weeks after the second intracameral injection (Fig. 1B). The patient has remained symptom-free 1 year after treatment. The final vision is 6/60 with a macular central corneal opacity. The patient at present awaits a penetrating keratoplasty.

Case 2

A 42-year-old male farmer presented with pain, watering, and diminution of vision in the right eye for 10 days, after minor trauma with a foreign body in a field. He was being treated by a general practitioner with 0.3% gentamicin drops but was gradually worsening. On examination, the vision was hand movements with accurate light projection in the right eye, which showed diffuse corneal edema, and a 2.5 × 3.5–mm corneal ulcer with feathery margins, extending as much as 60% to 70% in depth. There was a nonmobile hypopyon measuring 4.7 mm in height. The intraocular pressure was digitally high. Fundus details could not be seen and a B-scan US demonstrated a clear vitreous.

The corneal scraping revealed hyphae on a 10% KOH smear examination. Treatment was instituted with 5% topical natamycin every half hour, 0.3% ofloxacin drops four times daily, 1% atropine three times daily, and 250 mg of oral acetazolamide three times daily.

The patient showed no response to therapy after 7 days, and the hypopyon was found to have increased to 5.0 mm (Fig. 2A). Oral itraconazole 200 mg twice daily was added to the existing treatment. After 5 days, there was still no response, with the ulcer margins and the hypopyon progressing gradually. At this point, 0.15% topical amphotericin B was added to the previous regimen. The ulcer margins showed some rounding and the corneal edema decreased marginally, with clearing of the superficial stroma. However, there was no effect on the hypopyon.

The initial corneal scraping grew *A. flavus*. At the end of 2 weeks of therapy with topical amphotericin B, an aqueous tap was subjected to KOH smear and Calcofluor white stain examination and an intracameral injection of 10 μg of amphotericin B in 0.1 mL was given. The KOH wet mount examination of the aqueous fluid revealed hyphae.

Forty-eight hours after the intracameral injection, there was a dramatic improvement in the ulcer status. The surrounding corneal edema cleared, and the ulcer margins were rounded, measuring 2.1

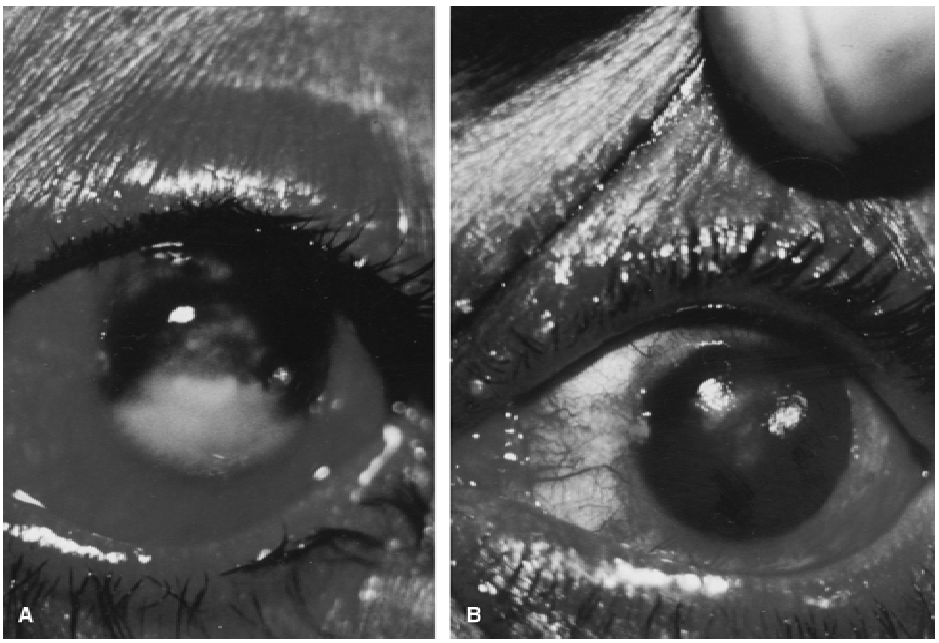


FIG. 1. A: Photograph of case 1 shows corneal ulcer and nonresponsive hypopyon. **B:** Photograph of the same patient 3 weeks after intracameral injection of amphotericin B shows resolution of the hypopyon.

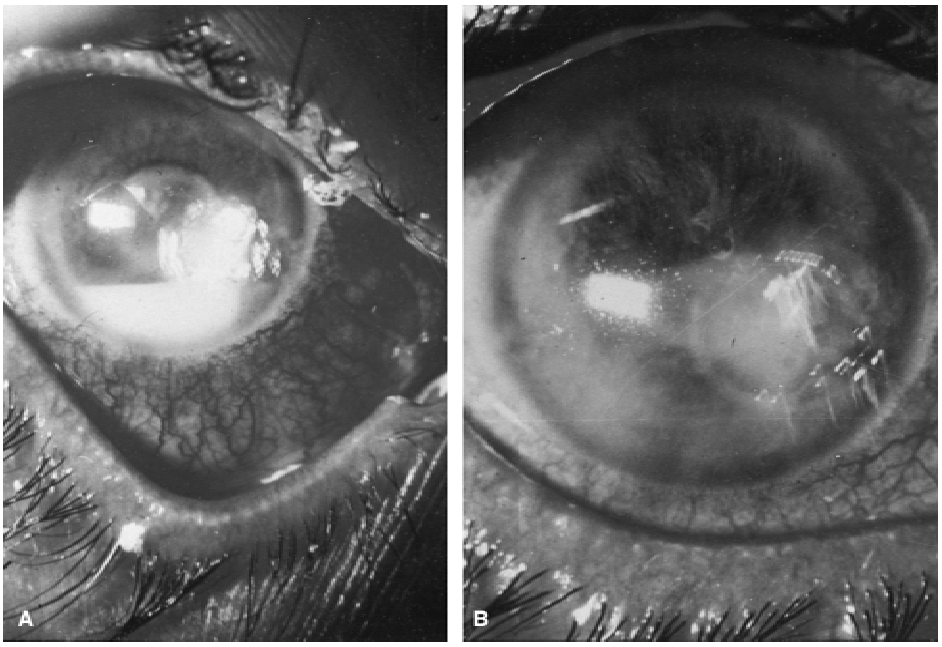


FIG. 2. A: Photograph of case 2 shows severe corneal ulcer with the resistant hypopyon. **B:** Photograph of the same patient 2 weeks after intracameral amphotericin B shows resolution of the hypopyon.

× 2.6 mm. The hypopyon had decreased for the first time in the course of the disease and now measured 3.1 mm. The rest of the treatment was continued. The aqueous sample also grew *A. flavus*. After responding well for 5 days, the ulcer became static. Intracameral amphotericin B 10 μg was repeated after 6 days of the initial injection. The ulcer and hypopyon responded well, and the infection was controlled 4 weeks after the final intervention (Fig. 2B).

Case 3

A 28-year-old healthy young man presented with a history of pain, redness, and watering in the left eye for 1 month. He gave no history of ocular trauma. A local practitioner was treating him with topical dexamethasone and chloramphenicol eyedrops. The condition worsened with therapy, and he was subsequently referred to the cornea service of our institute.

On presentation, the vision was 6/6 in the right eye and hand motions with accurate light projection in the left eye. There was a large corneal infiltrate measuring 5.5 × 5.0 mm with indistinct margins and raised edges involving nearly 90% of the corneal stroma. There was a nonshifting hypopyon measuring 2.8 mm. No posterior segment details were visible. The vitreous was clear on B-scan US. A corneal scraping was subjected to KOH wet mount examination, which revealed hyphae.

Treatment was started with 5% topical natamycin every hour, 200 mg oral itraconazole twice daily, 0.3% topical ofloxacin every 4 hours, 0.5% topical timolol twice daily, and 1% atropine drops three times daily. There was an initial response to treatment within 72 hours. The hypopyon reduced to 2.1 mm, the infiltrate cleared from the superficial stroma and reduced in size to 4.4 × 4.8 mm, and the margins started rounding. However, after 1 week of treatment, the infection was stationary, and there was no change in the hypopyon or posterior corneal infiltrate.

Topical amphotericin B 0.15% every hour was added to the existing regimen at this stage. One week of intensive treatment with daily scraping of the epithelium produced no significant benefit. At this stage, culture of the corneal scraping grew *A. flavus*.

The patient was then subjected to an aqueous tap and intracameral injection of 10 μg of amphotericin B in 0.1 mL. Hyphae were detected in the aqueous sample on KOH smear examination. The ulcer responded to treatment, the hypopyon decreased to 1.2 mm, and the size of the infiltrate reduced to 1.2 × 1.8 mm. Culture of the anterior chamber fluid yielded *A. flavus*.

The rest of the treatment was continued, and the ulcer and hypopyon steadily decreased. Two weeks after the intracameral injection, the infection was controlled, with the ulcer and hypopyon clearing completely. The vision in the affected eye was 6/24 with a paracentral macular corneal opacity.

Drug Preparation

Commercial preparations of amphotericin B (50 mg powder, Fungizone, Sarabhai Chemicals, Vadodara, India) for intravenous administration were diluted for intraocular injection. To achieve the desired concentrations, the drug was diluted aseptically in preservative-free sterile water for injection.

Method

Ten milliliters of distilled water for injection was added to the vial, giving a mixture containing 5.0 mg/mL; 1.0 mL of this was added to 4.0 mL of distilled water to further dilute it to 1.0 mg/mL. Of this concentration, 1.0 mL was then diluted with 9.0 mL of distilled water, thus making a concentration of 100 μg/mL; 0.1 mL of this contained 10 μg, which was injected with a 1.0-mL syringe into the anterior chamber. Similarly, 0.75 and 1.25 mL of this diluted drug contained 7.5 and 12.5 μg, respectively.

DISCUSSION

Amphotericin B was the first polyene effective in treating systemic mycosis.[6] This is the treatment of choice against yeasts and

natamycin-resistant filamentous fungi, notably *Aspergillus*.[15] Ocular penetration of the systemically administered drug into the eye is generally poor.[16] Pharmacokinetics studied in animals suggests that the corneal penetration of topically applied amphotericin B is poor in the presence of an intact corneal epithelium; however, in the absence of epithelium, the drug rapidly penetrates and reaches the anterior chamber.[17]

Natamycin has gained favor as an antifungal drug because of its wide antimycotic effect and relatively low toxicity. Additionally, it is the only U.S. Food and Drug Administration–approved antifungal agent currently available as an ophthalmic preparation. However, there are recent concerns regarding natamycin resistance and its inability to treat deep stromal keratitis. For these reasons, amphotericin B remains a potent agent in the treatment of keratomycosis. It has a wider spectrum of activity and, in addition, has immunoadjuvant properties and an immunopotentiating effect.[18] Improvement of the efficacy of amphotericin B may lie in its formulation and mode of application.

In an attempt to achieve optimal drug levels in the cornea, topical use of amphotericin B has been tried in the form of drops,[11] collagen shields,[19,20] and ointment.[21] The efficacy of topical amphotericin B drops has been studied clinically and experimentally using concentrations ranging from 0.003% to 0.3%.[7,17,22] Amphotericin B diluted to a concentration of 0.15% is efficacious in vivo and produces far fewer toxic effects than the higher concentrations previously used.[11]

To circumvent the problem of achieving adequate drug levels in the anterior chamber, subconjunctival and intraocular amphotericin B injections have been tried. Subconjunctival injections can produce long-standing periocular inflammation and can lead to epithelial ulcerations.[10] Experimental studies indicate little, if any, penetration into the aqueous by this route.[23] Intracameral and intravitreal injections have been used in the event of intraocular spread of infection. Although it has been widely used intravitreally for fungal endophthalmitis in doses of 5 to 10 μg in 0.1 mL,[13,24,25] the intracameral use has been restricted.[13,14,26,27]

In a rabbit model, Foster et al.[28] found that anterior chamber injection of as much as 50 μg amphotericin B failed to cause corneal or lenticular toxicity. The clinical dose recommended is 10 to 30 μg in 0.1 to 0.2 mL.[26] The first clinical report by Cutler et al.[27] describes two intracameral injections of 25 μg amphotericin B in a case of metastatic coccidioidal endophthalmitis. Pflugfelder et al.,[13] in their series of 19 cases of exogenous fungal endophthalmitis, described anterior chamber injections of amphotericin B in five patients. The dose most commonly used was 5 or 10 μg, and one patient with deep keratitis with total hypopyon received 50 μg.

No clinical evidence of corneal decompensation was noted in any patient even after repeated injections in some cases. Kermani and Aggarwal[14] treated a case of *A. niger* endophthalmitis with two injections of 5 μg intracameral amphotericin B in conjunction with oral itraconazole and reported resolution of the infection.

Clinical reports of intracameral amphotericin B for keratomycosis are few. In a case of *Colletotrichum graminicola* corneal ulcer,[12] intracameral amphotericin B was repeated twice in addition to repeated keratoplasties, and the infection was controlled.

The current report describes intracameral amphotericin B for cases of *A. flavus* keratitis with hypopyon not responding to topical natamycin or amphotericin B. Cases 1 and 2 developed a fungal ulcer secondary to trauma with vegetable matter, and case 3 had indiscriminate corticosteroid use. None of the patients responded to 5% topical natamycin given for more than a week. At the first sign of worsening, 0.15% topical amphotericin B and oral itraconazole was added in every case. Persistence with this regimen for at least 2 weeks with no sign of improvement prompted institution of intracameral amphotericin B. All three cases showed good response to 0.15% topical amphotericin B as regards the superficial ulceration, but the posterior corneal abscess and hypopyon persisted. Oral itraconazole was started in all three patients but was ineffective in clearing the hypopyon.

Intracameral amphotericin B was administered in all three patients. There was no clinical evidence of corneal or lenticular toxicity in any patient. Case 1 received two injections of 7.5 and 10 μg, case 2 received two injections of 10 μg each, and case 3 received one dose of 10 μg. All three patients showed good response to the drug, and the modality was instrumental in clearing infection that was otherwise resistant to other routinely used medical treatment.

Surgery in the form of a therapeutic penetrating keratoplasty has been advocated for deep keratitis with retrocorneal or anterior chamber involvement that is unresponsive to medical therapy.[8,13] However, results are often poor owing to the extensive infiltration of the anterior segment by the fungi. When fungal hyphae gain intracameral access, they are extremely difficult to eradicate.[10] Recurrence of the infection in the graft is common and often necessitates repeated grafting. In addition, intravitreal inoculation of fungi at the time of surgery is always possible leading to the catastrophe of fungal endophthalmitis. Intracameral amphotericin B could be a very useful modality to avoid surgical intervention at an acute stage of fungal keratitis.

The treatment of deep keratomycosis is complicated by the toxicity of antifungal agents irrespective of the route of administration. Intracameral amphotericin serves the purpose of drug delivery into the anterior chamber with none of the toxic features of high-concentration topical drops or systemic therapy. There are no clinically demonstrable untoward effects on the cornea with concentrations of 10 μg in 0.1 mL. Repeated doses can be given, depending on the response. This mode of therapy may assume greater significance in the emerging situations of resistance to natamycin owing to its widespread indiscriminate use.

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