Efficacy of Intracameral Amphotericin B Injection in the Management of Refractory Keratomycosis and Endophthalmitis

Safiye Yilmaz, MD, Melih Ture, MD, and Ahmet Maden, MD

Purpose: To evaluate the efficacy of intracameral amphotericin B injection in the adjunctive management of keratomycosis with probable intraocular extension not responding to conventional antifungal therapy.

Methods: Fourteen eyes of 12 patients with fungal keratitis that did not respond to initial treatment with topical and intravenous fluconazole and oral itraconazole were treated with up to 5 intracameral injections of 5 μg of amphotericin B. Six eyes received one injection, and 8 required subsequent injections.

Results: Twelve eyes responded to amphotericin B therapy, including 5 that healed with a central corneal scar and 8 that healed with a peripheral opacity. Two eyes progressed to evisceration. Four eyes developed anterior subcapsular cataract after intracameral amphotericin B.

Conclusions: Intracameral amphotericin B may be an effective adjunctive treatment of fungal keratitis unresponsive to conventional antifungal therapy, although cataract may occur.

Key Words: keratomycosis, treatment, intracameral injection, amphotericin B

Fungal keratitis accounts for 5%-20% of all corneal infections. The fungi are capable of penetrating the intact Descemet membrane and rapidly entering the anterior chamber. Hypopyon and inflammation of the membranes of the anterior chamber may easily develop during the course of the disease. In such cases, the conventional therapeutic options may not adequately treat fungi that have penetrated the endothelium even though adequate corneal concentrations of the drug may be attained. Amphotericin and natamycin are usually the first drugs of choice for fungal keratitis, but in the country in which this study was carried out, topical preparations of these drugs were not available. Systemic amphotericin B, which is well distributed in the choroidal circulation, has been used since 1959 in the medical management of keratomycosis, may also be used intravitreally for the treatment of endophthalmitis, and is safe if used at a reasonable dose. However, amphotericin B is not effective in eradicating fungi in the anterior chamber, because it penetrates poorly into the aqueous humor and may not reach adequate therapeutic levels. To try to successfully treat those fungi that had penetrated into the anterior chamber, we tested the use of intracameral injection of amphotericin B as an alternative to conventional therapies. The aim of this study was to evaluate the efficacy of intracameral amphotericin B injection in the management of keratomycosis with probable intraocular extension that has proven to be resistant to the conventional antifungal medical treatment.

MATERIALS AND METHODS

We examined 104 patients with a preliminary diagnosis of fungal keratitis who were referred to our institution between November 1999 and July 2004. This group included 12 patients who did not respond to topical and/or systemic antifungal treatment and thus were eligible to participate in a study to examine the efficacy of intracameral amphotericin B administration. The research protocol used in this study was approved by the Training and Planning Committee of the institution, and informed consent was obtained from all subjects after an explanation of the nature of and possible consequences of the study.

All patients were examined in the same clinic at our institution, and complete histories were obtained from each patient, after which they underwent a slit-lamp microscopic examination. Corneal cultures and scrapings were obtained from the corneal ulcer, and the cultures were transferred into a transport medium by use of a sterile cotton-tip swab. The corneal scrapings were placed on a glass slide, and together with the cultures, were taken to the microbiology laboratories. Because intrinsic technical limitations prevented us from obtaining results consistently from the cultures, we relied more heavily on the results of the microscopic analysis of the corneal scrapings.

The study examined 14 eyes of 12 patients, all of whom exhibited hypopyon and/or inflammation of the anterior chamber membranes. Before treatment with intracameral injection of amphotericin B, all patients were hospitalized and received 0.3% topical fluconazole drops once an hour as a first line of
treatment. If these patients subsequently developed an increase in the infiltrate size, enlargement of the endothelial plaque, or hypopyon, intravenous fluconazole and oral itraconazole were added to the treatment regimen and continued for 14 days. However, none of the patients responded to the fluconazole and itraconazole regimen, necessitating treatment with an intracameral injection of amphotericin B, which was prepared as indicated by Kaushik et al. Ten milliliters of 5% dextrose was added to a vial containing 50 mg of amphotericin B, and 0.1 mL of this solution was diluted to 10 mL with 5% dextrose, resulting in a final solution in which a 0.1-mL dose contained 5 μg of amphotericin B.

The injection procedure was carried out by the same surgeon who had carried out the initial treatments (S.Y.) in an operating room after topical anesthesia. Before surgery, the eye was cleaned by a standard method using povidone iodine. A limbal incision was made with a no. 11 surgical blade at the clear corneal sides, the endothelial plaque region was gently aspirated, and an aqueous tap was performed. The contents of the tap were subsequently inoculated on Sabouraud dextrose agar. A total of 0.1 mL of 5% dextrose solution containing 5 μg of amphotericin B was intracameraly injected using a cannula on a tuberculin syringe.

Repeat injections containing the same dose (5 μg of amphotericin B) were performed as necessary, on the basis of the clinical response. Repeat injections were scheduled for all of the patients until the endothelial plaque disappeared or until the treatment was deemed to have failed. The interval between repeat injections was dictated by how quickly the local inflammatory response resulting from the previous injection subsided; time between injections varied from 1 to 10 days depending on the patient. These repeat injections were given using the same paracentesis site for each intervention. Treatment success was defined as resolution of the corneal infiltrate, disappearance of the endothelial plaque, and healing of the epithelial defect.

RESULTS

The mean age of the patients was 50.2 ± 13.5 (SD) years (range, 32–67 years). Seven patients were men, and 5 patients were women. Six (50%) of the 12 patients had a history of diabetes mellitus, and bilateral involvement was seen in 2 (33.3%) of the 6 patients with diabetes within 2 months after the first eye involvement. The remaining 6 patients without diabetes (50%) had a history of foreign body injury. Seven patients, 2 of whom were contact lens wearers, had previously received topical steroid therapy at another clinic. Two patients had bilateral infection. They were diabetic, and 1 of them was also a farmer, and the other was also a contact lens user.

In the 14 eyes in the study group, laboratory test results showed a positive stain and/or culture for the presence of fungus. Six eyes had a positive stain but not a positive culture, and 8 eyes had a positive stain and a positive culture. Of the 6 eyes with a positive stain only, 2 scrapings showing hyphae were molds and 4 scrapings showing pseudohyphae were yeast. Of the other 8 eyes with positive cultures, 4 scrapings grew Aspergillus spp., 2 grew Fusarium spp., and 2 were identified as Candida albicans. Of the 2 patients with bilateral infection, 1 had positive culture from both eyes that was identified as C. albicans, the second patient had positive stain from both eyes, and the scrapings showed pseudohyphae. The hypopyon in these cases of deep keratomycosis generally contained fungal elements: 8 showed hyphae and 6 showed pseudohyphae. Of the 4 eyes in which hypopyon yielded positive cultures, 2 of the cultures grew C. albicans and 2 grew Aspergillus spp.

In all eyes, an extensive corneal ulcer exhibiting hypopyon was present before the eyes were treated with intracameral injection of amphotericin B. The fundus details could not be clearly discerned, because of the corneal infiltrate and/or the anterior chamber reaction, although B-scan ultrasonography showed a clear vitreous. Four eyes in 4 patients were pseudophakic. After initial treatment with 0.3% topical fluconazole failed, treatment was continued with administration of systemic intravenous fluconazole and oral itraconazole for at least 14 days in all patients. After initiation of fluconazole and itraconazole therapy, the average follow-up time before performing the first intracameral amphotericin B injection was 17 ± 4.77 days; both topical and systemic antifungal therapies were continued during the course of successive intracameral injections.

Although 6 eyes healed after just 1 intracameral injection, 8 eyes needed subsequent injections. Of these 8 eyes, 3 eyes required 2 injections, 1 eye required 3 injections, and 4 eyes required 5 injections. Of the 8 eyes that received a second injection, 3 eyes responded well, but 5 eyes were given further injections for at most 5 times during a period that ranged from 11 to 34 days. One eye responded well enough that the infection was controlled 12 days after the third intervention, and in 1 eye, the infection was controlled 30 days after the fifth intervention. One patient refused the repeat injections after 3 doses because of the pain experienced; in this patient, the corneal ulcer did eventually heal in the absence of further intervention by injection, although conventional treatment was continued after the injections were discontinued. However, in 2 eyes, the corneal ulcer became worse despite the 5 intracameral injections, and the eyes ultimately had to be therapeutically enucleated.

In all cases, regardless of the ultimate outcome, hypopyon disappeared between 2 and 10 days after the last injection (mean, 6.07 ± 2.07 days). In those cases in which the corneal ulcer was resolved, complete resolution occurred between days 21 and 60 (mean, 32.14 ± 11.67 days). Five eyes healed with a peripheral corneal scar (Fig. 1), 7 eyes healed with a mild, central corneal scar (Fig. 2), and 2 eyes required enucleation, as described above. Six months after the last injection, 3 patients with a central corneal scar underwent a corneal transplant. The final visual acuity depended on the location of the remaining scar. Visual acuities were measured with Snellen line in all patients. Visual acuity of all patients ranged between hand motion and 1 Snellen line before the treatment with intracameral amphotericin B. In 11 eyes, vision improved at least 1 Snellen line after the treatment. In the other 3 eyes, vision did not improve. Mean improvement for all patients was 1.7 (range, 1–6) lines of Snellen acuity after the treatment. No eye lost one line of Snellen acuity except for the patients with enucleated eyes.
At the time of the baseline examination (day 1), all eyes were negative in terms of cataracts, vitreal opacities, and corneal toxicity. Anterior subcapsular cataracts were observed in 4 (28.5%) of 14 eyes between days 18 and 60 (mean time of appearance, 38.28 ± 13.22 days) after the final injection. All the eyes that developed cataracts received 1 repeat injection of amphotericin B; depending on the patient, there were 2 to 4 extra injections. At the final examination of the patients, the eyes did not exhibit vitreous opacity or band formation, and there was no evidence of either local or systemic toxic side effects. In all of the successfully treated eyes, there was no recurrence of the infection after withdrawal of all of the antifungal agents.

Six months after the last injection, we performed cataract extraction and intraocular lens implantation for 4 eyes and penetrating keratoplasty for 3 eyes with extensive central corneal scar.

**DISCUSSION**

Fungal keratitis is a significant cause of ocular morbidity in rural areas where the climate is warm and humid. The 2 most common forms of fungi that are of interest to ophthalmologists are filamentous fungi and yeast. Although treatment of ophthalmic fungal infections has improved with the development of new broad-spectrum antimycotics, cases of severe infection caused by antifungal-resistant fungi have...
increased recently, making successful treatment more difficult. In this context, the selection of appropriate therapy for fungal infection remains an unsettled issue. The clinical efficacy of any antifungal agent used for therapy for fungal keratitis depends greatly on the final concentration achieved in the target corneal tissue, on its spectrum of activity, and on its safety profile. As a result, treatment with a variety of antifungal agents is complicated by a narrow spectrum of activity, lack of effective penetration into the eye, and toxicity.10,11

Amphotericin B is the first polyene compound that has been shown to be effective in treating systemic mycosis and is the treatment of choice against yeasts and natamycin-resistant filamentous fungi, notably *Aspergillus*.11,12 The efficacy of amphotericin B is closely related to the ability to achieve optimal drug levels in the cornea, but clinicians are often reluctant to administer intravenous amphotericin B because of various side effects including nausea, vomiting, and renal dysfunction. The risk of these side effects is decreased if the drug is introduced by another route such as topical, subconjunctival, or intravitreal.6,13 However, even if it were possible to achieve an adequate corneal concentration by the use of topical drops, the administration may not successfully treat the organisms that have already penetrated the endothelium.13 Therefore, direct intravitreal and intracameral injection of amphotericin B is indicated for the treatment of fungal endophthalmitis because of the poor intracameral concentrations obtained with intravenous administration and because of concerns about the nephrotoxicity that are associated with systemic exposure to amphotericin B.15 Fungal hypopyon, which frequently occurs in cases of fungal keratitis, is particularly difficult to treat because the ability of most topically applied drugs to penetrate the cornea is poor in the presence of an intact corneal epithelium. The exact role of amphotericin B in the healing of fungal keratitis is not clear. We think that the topical drugs treated only the fungal keratitis and not intraocular extension of fungal infection. On the other hand, the intracameral injection of amphotericin B cured the intraocular extension and the corneal infection that has penetrated the endothelium.

In contrast to the intracameral mode of delivery used in this study, other studies have used intravitreal administration of amphotericin B for the treatment of fungal endophthalmitis. Cannon et al15 reported that the administration of intravitreal amphotericin B was generally well tolerated and recommended doses ranging from 5 to 10 μg in 0.1 mL. However, an in vitro study showed that intracameral amphotericin B is toxic to the corneal endothelium.16 Only a few studies have reported data concerning the intracameral application of amphotericin B in the management of keratomycosis, and most of them were experimental laboratory studies and not clinical studies.15-20 To our knowledge, no clinical study examining intracameral amphotericin B therapy and including ≥5 patients has previously been reported in the literature, so the 12-patient, 14-eye study that we report here is the first of its scale. Results from previous laboratory or small-scale clinical studies have shown that amphotericin B injection is generally safe; Foster et al16 found that, in a rabbit model, anterior chamber injection of as much as 50 μg amphotericin B failed to cause corneal or lenticular toxicity. An initial clinical report by Cutler et al18 described 2 intracameral injections of 25 μg of amphotericin B in a patient with metastatic coccidiodial endophthalmitis, and Pfugfelder et al17 performed anterior chamber injection of either 5 or 10 μg of amphotericin B in 5 patients with exogenous fungal endophthalmitis.

In this study, we described the administration of intracameral amphotericin B for cases of *Aspergillus* spp., *Fusarium* spp., and *C. albicans* keratitis that exhibited hypopyon and did not respond to conventional topical and systemic fluconazole and itraconazole therapy. Of the 12 participants, 6 eyes had a positive stain but yielded no fungal growth on culture. Two possible causes could be considered for this result; first, the laboratory is not in our clinic and it takes time to send the specimen to the laboratory. Also, we do not know how long the specimens wait in the laboratory. Therefore, the unviable fungus might be seen on scratchings but they did not grow on culture. Second, some patients with fungal keratitis were referred to our institution after topical treatment. As a result of this treatment, the specimens taken were already unviable.

Treatment with the standard topical antifungal agent 5% natamycin or topical amphotericin B could not be provided because these preparations are currently unavailable in our country. In the cases described here, persistent treatment with systemic and topical antifungal agents for ≥2 weeks produced no signs of improvement, prompting the use of intracameral injections of amphotericin B. After injection, the first sign of improvement was reduced infiltrate size and/or decreased hypopyon level, and in successfully treated cases, the hypopyon ultimately disappeared between 2 and 10 days after the last injection.

Injection of amphotericin B has generally been found to be safe and effective: In 2 other studies, no corneal decompensation was reported after repeated injections of amphotericin B.19,20 Kermani and Aggarwal14 treated a case of *Aspergillus niger* endophthalmitis with 2 intracameral injections of 5 μg of amphotericin B in conjunction with oral itraconazole and reported successful resolution of the infection. In a case of *Colletotrichum gramincola* corneal ulcer, intracameral amphotericin B was repeated twice (in addition to repeated keratoplasties), and the infection was controlled.20 Kaushik et al8 reported the use of intracameral amphotericin B in 3 patients with culture-verified *Aspergillus flavus* corneal ulcers and associated hypopyon and found that all 3 patients responded favorably to 1 or 2 injections at doses of 7.5 or 10 μg, with complete clearing of corneal ulcer and hypopyon and no evidence of corneal or lenticular toxicity. In another case series by Kuriakose et al,21 4 patients with deep keratomycosis underwent repeated (3–13 times) injections of intracameral amphotericin B, with 5 μg in 0.1 mL of 5% dextrose administered in each injection over 6 to 36 days. Three of the 4 patients had complete resolution of the ulcer, although 1 patient perforated and required evisceration.

In this study, we used intracameral injection of 5 μg of amphotericin B for fungal keratitis with probable intraocular extension, the same dose as reported by Kaushik et al8 and Kuriakose et al.21 In our study, 6 patients needed only 1 injection of amphotericin B and 8 patients required repeated injections (up to 4) for successful resolution of the infection. The patients who needed repeat injections had a preexisting
history of diabetes mellitus or ocular trauma as the etiology of the keratitis. Three patients had extensive corneal scarring after the resolution of the infection. Three patients, 4 eyes, in which the keratitis and the associated anterior chamber inflammatory reaction were completely resolved with the repeated intracameral administration of amphotericin B ultimately developed a cataract. The cause of the cataract in these patients is unclear, although possible causes include amphotericin B toxicity, an inflammatory reaction, and/or injection trauma. This cataract development was probably related to the involvement of the anterior capsule of the lens in the infection or to the cause of accidental injury to the lens because of the extreme inflammation in the anterior chamber, which led to the disappearance of the lens surface.

Our study had some limitations: We did not assess the efficacy of the ideal concentration of amphotericin B by testing a dosage curve, and we did not examine different time intervals between injections, nor did we evaluate different amphotericin B formulations with regard to toxicity. In this respect, on the basis of ophthalmologic data,19,21 the 5-μg dose that we used in the study was shown to be safe and effective for the treatment of fungal corneal ulcer with hypopyon. Nevertheless, further studies evaluating the efficacy of using higher doses of amphotericin B for treatment of eyes that did not respond to repeated injections at the 5-μg level should be performed. However, the 5-μg dose clearly does have clinical efficacy, because our results showed that 6 eyes healed after just one 5-μg intracameral injection, and 3 eyes healed after two 5-μg injections. In the 3 eyes that required >3 repeated injections, only 1 eye responded to the therapy, and 2 required evisceration. Therefore, our data suggest that 1 or 2 intracameral injections of 5 μg of amphotericin B may be generally sufficient for the management of refractory keratomycosis, thus avoiding complications related to possible toxicity that might result from increased doses of intracameral amphotericin B.

Results from this study show that the use of intracameral injection of amphotericin B as a treatment of deep keratomycosis with hypopyon allows the clinician to avoid keratoplasty as a primary mode of treatment. Avoidance of this surgery is beneficial on several counts, because 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 and may lead to catastrophic fungal endophthalmitis. Intracameral amphotericin B may be a useful treatment of the acute stage of fungal keratitis in that it is generally efficacious and allows the clinician to avoid surgical intervention. However, the risk of anterior chamber inoculation of fungal organisms after an intraocular injection needs to be seriously considered. To minimize this risk, the injection procedure should be performed under strict aseptic conditions. In addition, because there is poor visibility of the anterior chamber structures, care should be taken to avoid injury to the crystalline lens, and the possibility of subsequent cataract formation should be explained to the patient before his or her decision to undergo injection therapy.

In conclusion, results from this study showed that intracameral amphotericin B injection could be a useful alternative treatment of severe fungal keratitis with intraocular extension that is resistant to conventional therapy. The intracameral injection of amphotericin B readily delivers a therapeutic concentration to the anterior chamber without significant adverse effects, although there is a caveat: As the injection of amphotericin B is being performed, the clinician must be careful to avoid inoculation of the anterior chamber with fungal organisms and to avoid lenticular injury that could result in cataract formation.

REFERENCES