Comparison of Topical Itraconazole 1% With Topical Natamycin 5% for the Treatment of Filamentous Fungal Keratitis

C. M. Kalavathy, MS, Pragya Parmar, MS, J. Kaliamurthy, PhD, Vinitha Rachel Philip, DNB, M. D. K. Ramalingam, MS, C. A. Nelson Jesudasan, MS, and Philip A. Thomas, MD, PhD

Purpose: To compare the clinical efficacy of itraconazole 1% eyedrops with a standard therapy regimen (natamycin 5% eyedrops) for topical monotherapy of fungal keratitis.

Methods: Patients presenting with suspected uniocular microbial keratitis over a period of 12 months (January to December 2002) underwent detailed clinical examination and microbiological investigation. One hundred consecutive patients with direct smear- and/or culture-proven fungal keratitis were enrolled in the study after obtaining informed consent. The ulcers were categorized as severe or nonsevere. The first 50 consecutive patients received primary therapy with topical natamycin hourly, and the next 50 consecutive patients received topical itraconazole hourly. The primary efficacy criteria were the physician's judgment of clinical success, cure rate, and the rate of treatment failure.

Results: The diagnosis of fungal keratitis was established by positive microscopy and culture findings in 88 patients and by positive microscopy alone in 12 patients. Species of *Fusarium*, *Aspergillus*, and *Curvularia* were the principal isolates. Thirty-six (72%) of 50 patients (28 of 37 with nonsevere keratitis and 8 of 13 with severe keratitis) showed a favorable response to primary natamycin therapy (mean duration, 20.5 days), while 30 (60%) of 150 patients (25 of 38 with nonsevere keratitis and 5 of 12 with severe keratitis) exhibited a favorable response to primary itraconazole therapy (mean duration, 23.1 days). In keratitis due to *Fusarium* spp, 19 (79%) of 24 patients showed a favorable response to natamycin, which was significantly greater than the 8 (44%) of 18 patients who showed a favorable response to itraconazole ($P < 0.02$). However, no such difference was evident in keratitis due to *Aspergillus* spp or *Curvularia* spp; in keratitis due to *Aspergillus* spp, favorable responses were noted in 6 (54.5%) of 11 patients receiving natamycin and 5 (50%) of 10 patients receiving itraconazole, while in keratitis due to *Curvurlaria* spp, such responses occurred in both patients receiving natamycin and in 8 (89%) of 9 patients receiving itraconazole. Both antifungal formulations were generally well tolerated with no obvious adverse effects.

Conclusions: Topical natamycin should continue to be considered as the treatment of choice for filamentous fungal keratitis; when natamycin is unavailable, topical itraconazole therapy could be used, particularly if the infections are due to *Aspergillus* or *Curvularia* spp.

Key Words: itraconazole, natamycin, keratitis, fungal, therapy

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Fungal infections of the cornea are important causes of ocular morbidity and even blindness in the developing world. Hyaline filamentous fungi, such as *Aspergillus* spp and *Fusarium* spp, and dematiaceous fungi, such as *Curvularia* spp, are the most important causes of such infections in tropical and subtropical regions.[1–3] The therapy for fungal keratitis is currently hampered by the limited availability of effective antifungal drugs for topical administration.[4] The polyene natamycin (pimaricin), the first antifungal compound approved by the U.S. Food and Drug Administration for topical ocular use, is available as a 5% suspension for topical administration.[5] It has been recommended and is used wherever available as the drug of choice for first-line treatment of filamentous fungal keratitis.[1,4–6] The synthetic bistriazole fluconazole exhibits outstanding pharmacokinetic properties in the eye[4,5] and is clinically effective in *Candida* keratitis[7] but is inferior to natamycin as primary therapy in filamentous fungal keratitis.[8] Recently, a 2% preparation of econazole for topical application was reported to be as effective as natamycin 5% in the management of filamentous fungal keratitis.[9] However, there is a need to evaluate other topical antifungals.

Itraconazole, a synthetic dioxolane triazole, is well absorbed after oral administration[4] and has pronounced in vitro antifungal activity against a wide range of fungi, including *Aspergillus* spp and *Curvularia* spp.[5] Oral itraconazole (200 mg/d), while effective as therapy for nonsevere fungal keratitis,[10] is less so in severe keratitis, possibly because of its poor penetration of the eye.[11] Guzek et al[12] demonstrated that when itraconazole was dissolved in a suitable vehicle and applied topically to rabbit corneas, approximate itraconazole concentrations of 200 to 250 μg/g corneal tissue were achieved. We sought to evaluate the potential clinical efficacy of a commercially available itraconazole 1% eyedrop preparation by comparing it with a standard therapeutic regimen (natamycin 5% eyedrops) as therapy for fungal keratitis.

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From the Institute of Ophthalmology, Joseph Eye Hospital, Tamil Nadu, India.
Reprints: C. M. Kalavathy, MS, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirappalli 620001, Tamil Nadu, India (e-mail: cmkala@eth.net).

MATERIALS AND METHODS

Patients who presented with clinical features suggestive of microbial keratitis at the Cornea Clinic of Joseph Eye Hospital, Tiruchirapalli, India, between January 2002 and December 2002 underwent microbiological investigations by standard protocols.[13,14] Briefly, material was scraped from the base and edges of each corneal ulcer by a sterile, blunt cataract knife. Some material was used to prepare lactophenol cotton blue-stained wet preparations (for detection of fungi and *Acanthamoeba* cysts)[14,15] and Gram-stained smears for direct microscopic examination.[13,14] Corneal material was also inoculated in rows of C streaks onto plates of 5% sheep blood agar (incubated at 25° and 37°C for isolation of fungi and bacteria, respectively), cystine tryptone agar (incubated at 37°C for bacterial isolation), and Sabouraud glucose neopeptone agar (incubated at 25° and 37°C for isolation of fungi) and into bottles of thioglycollate medium (for isolation of anaerobic organisms). Bacterial and fungal isolates were identified by standard methods.[4,13] One hundred consecutive patients with either direct smear-positive and/or culture-proven fungal keratitis were enrolled in the study after obtaining prior informed consent. Patients with mixed infections, those in whom direct microscopy and culture did not yield significant findings, and those who had previously received antifungal therapy were not enrolled. The study received prior approval from the Institutional Review Board of this institute.

Prior to the initiation of antifungal therapy, the patients were subjected to detailed slit-lamp evaluation to determine the minimal and maximal dimension of the epithelial defect and infiltration. Each ulcer was categorized as nonsevere and severe based on previously described criteria[13]; a nonsevere ulcer had a diameter ≤6 mm, ulceration of the superficial one third, and suppuration of the superficial two thirds of the corneal layers, while a severe ulcer had a diameter >6 mm with ulceration and suppuration involving the deep one third of the cornea.

The commercial natamycin ophthalmic preparation is packaged as a suspension and precipitates in the corneal tissue following topical administration; these precipitates are obvious to the examining ophthalmologist. Hence, it was deemed not possible to perform a truly double-masked, randomized clinical trial. Instead, the first 50 consecutive patients (26 males and 24 females ranging in age from 10 to 55 years), who constituted group 1, received topical applications of natamycin 5% eyedrops hourly (Natamet, Milmet, Mumbai, India) following smear or culture confirmation of the diagnosis. The next 50 consecutive patients (34 males and 16 females, ranging in age from 17 to 67 years), who constituted group 2, received topical hourly applications of itraconazole 1% eyedrops (Itral, Jawa, Mumbai, India). The patients were examined daily by the investigating ophthalmologist to assess ulcer healing (graded as completely healed, healing, worse, or no change) and percentage change from base line (increase or decrease) in fluorescein staining and infiltrate. If there was obvious clinical improvement, treatment was continued until the ulcer had healed fully, and then gradually tapered and stopped. If there was no clinical improvement or if there was progressive ulceration, treatment was discontinued and other measures initiated. All patients received adjunctive therapy, consisting of ciprofloxacin 0.3% eyedrops, mydriatics, and systemic analgesics. The patients were also monitored for signs and symptoms suggestive of an adverse event. The χ^2 test and Student t test were used to assess the statistical significance of certain results.

RESULTS

The baseline demographic characteristics of the individuals in the 2 treatment groups, as also the ulcer parameters, were essentially similar (Table 1). Thirty-seven (74%) of 50 patients in group 1 and 38 (76%) of 50 patients in group 2 presented with nonsevere ulcers. The most commonly isolated fungal pathogen in both treatment groups was *Fusarium* (48% in group 1 and 36% in group 2). The mean duration of treatment was 20.5 ± 10 days in group 1 (primary natamycin therapy) and 21.3 ± 9.5 days in group 2 (primary itraconazole therapy); this difference was not statistically significant (t = 0.17, $P > 0.05$).

In 36 (72%) of 50 patients in group 1 (receiving primary treatment with natamycin) and in 30 (60%) of 50 patients in group 2 (receiving initial treatment with topical itraconazole), the corneal lesions resolved completely or exhibited definite signs of healing (Table 2), and this was deemed to be a favorable response; however, this difference was not statistically significant (χ^2 (df = 1) = 1.6; $P > 0.05$). Eight (61%) of 13 severe ulcers healed with primary natamycin therapy, whereas only 5 (42%) of 12 severe ulcers healed with primary itraconazole therapy (Table 2); again, this difference was not statistically significant.

In keratitis due to *Fusarium* spp, 19 (79%) of 24 patients showed a favorable response to primary natamycin therapy, in contrast to 8 (44%) of 18 patients who responded to topical itraconazole therapy (Table 3); this difference was statistically significant (χ^2 (df = 1) = 6.9; $P < 0.02$). In keratitis due to

TABLE 1. Characteristics of Patients With Fungal Keratitis Enrolled in the Study

Parameter	Group 1 (Natamycin-Treated) (n = 50)	Group 2 (Itraconazole-Treated) (n = 50)
Age, y (mean)	10–53 (38.18)	17–67 (39.8)
Gender		
Female	24	16
Male	26	34
Severity		
Nonsevere	37	38
Severe	13	12
Isolated fungal pathogens		
Fusarium spp	24	18
Aspergillus spp	11	10
Curvularia spp	2	9
Other fungi	10	4
No growth of fungi*	3	9
Mean duration of therapy (d)	20.5	23.1

*These ulcers were smear positive for fungi but culture negative.

TABLE 2. Correlation Between the Severity of Ulcer and Response to Therapy in the 2 Groups of Patients With Fungal Keratitis

Response to Therapy	Group 1		Group 2	
	Severe	Nonsevere	Severe	Nonsevere
Healed/healing	8	28	5	25
Poor	5	6	7	11
No change	0	3	0	2
Total	13	37	12	38

Severity of keratitis based on definitions by Jones.[13]
Group 1, treated with topical natamycin 5%; group 2, treated with topical itraconazole 1%.

Aspergillus spp, 6 (54.5%) of 11 patients showed a favorable response to primary therapy with topical natamycin 5% compared with 5 (50%) of 10 patients who had received primary treatment with itraconazole; this difference was not statistically significant. In keratitis due to *Curvularia* spp, 2 (100%) of 2 patients showed a favorable response to primary natamycin therapy when compared with 8 (89%) of 9 patients who responded to topical itraconazole therapy.

No adverse events were recorded in any of the individuals in either treatment group.

DISCUSSION

The clinical efficacy of any antifungal agent used for therapy for fungal keratitis depends greatly on the concentration achieved in the corneal tissue, its spectrum of activity and its safety profile.[4,16] Amphotericin B was the treatment of choice for fungal keratitis for many years since it satisfied the first 2 requirements.[5] However, its toxicity and other limitations led to its slow replacement by natamycin as the drug of choice for filamentous fungal keratitis. Natamycin possesses certain attributes that make it ideal for treatment of filamentous fungal keratitis. It has a broad spectrum of antifungal activity, especially against *Fusarium* spp, which are the most common causes of fungal keratitis in many parts of the world[1–5]; it is available as a topical ophthalmic preparation that is stable and adheres well to the cornea for clinically useful periods; although viscous, it is well tolerated and causes no pain or secondary corneal damage.[17] However, natamycin barely penetrates an intact corneal epithelium because diffusion is increasingly reduced due to its molecular mass (665.75 Da).[16] Poor ocular penetration is believed to be another drawback of topical natamycin therapy,[17] which has led some workers to use topical natamycin monotherapy only in superficial fungal keratitis and to combine topical natamycin therapy with oral azole therapy in deep (severe) keratitis.[1] As long ago as the 1970s, Jones et al[18] had advocated the use of topical saturation therapy when treating fungal keratitis, with a view to achieving effective therapeutic concentrations of topical antifungals in the corneal tissue. Radiolabeling studies performed in 1986[19] support this concept of the value of a loading dose when using topical antifungals. In these studies, 13 topical applications every 5 minutes resulted in a drug concentration of approximately 2.5 mg/g cornea in rabbit corneas debrided of epithelium; levels peaked at approximately 10 minutes after administration. Far lower levels (7.0 μg/g) were attained in corneas in which the epithelium was left intact.[19] Although it is unclear whether these levels are actually achieved during therapy for clinical mycotic keratitis, these data encouraged us to use topical natamycin as primary monotherapy even in severe keratitis; healing was achieved in 61% of such ulcers.

In recent years, azole compounds have shown tremendous promise in the therapy for fungal keratitis[4,5] but have not really superseded natamycin as the treatment of choice in filamentous fungal keratitis. In a small series of patients, Rao et al[8] demonstrated that primary fluconazole therapy was decidedly inferior to natamycin for filamentous fungal keratitis. Interestingly, Prajna et al[9] recently reported that a topical econazole 2% preparation was as effective as topical natamycin for management of fungal keratitis; however, the results were not evaluated with reference to severity of the ulcers or the species of fungi isolated.

Itraconazole has a broad spectrum of antifungal activity.[4,5] Being lipophilic, it easily crosses the lipid-rich corneal epithelium and endothelium but has limited penetration of the corneal stroma. However, a suitable vehicle may improve the corneal penetration of itraconazole.[12] Pankaj et al[20] evaluated the efficacy of topical 1% and systemic itraconazole in 54 patients with fungal keratitis and a favorable response was obtained in 77% of cases; here again, the outcome in relation to severity of keratitis and the infecting fungus was not clearly

TABLE 3. Correlation Between the Type of Fungus and Response to Therapy in 2 Groups of Patients With Fungal Keratitis

Species of Fungus	Response to Therapy in Group 1				Response to Therapy in Group 2			
	Total	Healed/Healing No. (%)	Worse	No Change	Total	Healed/Healing No. (%)	Worse	No Change
Fusarium	24	19 (79)	4	1	18	8 (44)	10	—
Aspergillus	11	6 (54.5)	5	—	10	5 (50)	3	2
Curvularia	2	2 (100)	—	—	9	8 (89)	1	—
Other	10	6 (60)	2	2	4	2 (50)	2	—
No growth*	3	3 (100)	—	—	9	7 (77)	2	—
Total	50	36 (72)	11	3	50	30 (60)	18	2

*These ulcers were smear positive for fungi but culture negative.
Severity of keratitis based on definitions by Jones.[13]
Group 1, Primary treatment with topical natamycin 5%; group 2, Primary treatment with topical itraconazole 1%.

stated, nor was the source of the topical itraconazole preparation mentioned. In the present study, itraconazole was dissolved in sufficient quantity of sterile aqueous base containing benzalkonium chloride solution (0.02% vol/vol). Hourly topical application of this preparation resulted in favorable responses in 60% of cases (42% of severe ulcers responded to primary topical itraconazole therapy).

In our study, *Curvularia* spp constituted the third most frequent cause of mycotic keratitis. In a study by Wilhelmus and Jones,[21] 35 (83%) of 42 patients with *Curvularia* keratitis responded to antifungal alone (principally natamycin in 27 and other antifungals in remaining 8 patients). In our study, 10 (91%) of 11 patients (natamycin in 2 patients and itraconazole in 8 patients) showed a favorable response to treatment. Hence, while advocating the use of natamycin as first-line therapy for keratitis due to *Curvularia* spp, we suggest that itraconazole could be considered as an effective alternative should natamycin be unavailable or ineffective.

In this study, we have compared the efficacy of topical preparations of natamycin and itraconazole in treatment of filamentous fungal keratitis. No adverse effects were reported from either treatment group. Precipitation of the natamycin eyedrops did occur following topical application, but this did not impair healing of the ulcers. In fact, the paste-like suspension may have actually prolonged the contact time of the compound with the corneal tissue and reduced the effects of dilution of the antifungal by the tears present in the cul-de-sac.[5] We observed that in patients with *Fusarium* keratitis, a significantly higher proportion responded to topical natamycin than to topical itraconazole. Overall, a higher proportion of patients treated with natamycin exhibited favorable responses than those receiving topical itraconazole. The mean duration of treatment was marginally longer in the itraconazole-treated group than in the natamycin-treated group, but this was not statistically significant ($P > 0.05$; $t = 0.17$). These results suggest that topical natamycin should continue to be considered as the therapy for choice for filamentous fungal keratitis; when not available, topical itraconazole therapy could be used, particularly if the infections are due to *Aspergillus* spp or *Curvularia* spp.

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