Oral Itraconazole Therapy for Mycotic Keratitis
Orale Itraconazol-Therapie bei mykotischer Keratitis

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Summary: Forty consecutive patients suffering from mycotic keratitis (19 due to Fusarium solani and other Fusarium spp., 15 due to Aspergillus flavus and Aspergillus fumigatus and six cases due to other fungi) were treated with itraconazole, a triazole derivative. The compound was administered orally once daily in a dose of 200 mg for a median duration of treatment of 17 days. Progressive corneal ulceration stopped and there was complete resolution of all lesions and eradication of the infecting fungus from the lesions in 22 patients. In five patients, the infecting fungi were eradicated from the lesions but ultimately surgery had to be performed due to incomplete resolution of the lesions. In the remaining 13 patients, progressive corneal ulceration continued and the infecting fungi (F. solani and other Fusarium spp. in nine patients, A. fumigatus in two patients, A. flavus in one and Cladosporium spp. in one patient) were not eradicated from the lesion. Excellent or moderate responsiveness to therapy was observed more frequently in cases of keratitis due to Aspergillus than in cases of keratitis due to Fusarium. There was no evidence of serious adverse reactions in any of the patients.


Introduction
Itraconazole (R 51 211, Janssen Pharmaceutica, Belgium) is a new, orally-active, triazole derivative with a broad spectrum of antifungal activity. Espinel-Ingroff et al. (4) and Van Cutsem et al. (15) have shown this
compound to have pronounced antifungal activity against a wide range of fungi, especially various species of *Aspergillus*. In animal models of aspergillosis, cryptococcosis and other fungal infections, this drug has been found to be more potent than ketoconazole (16). This drug has been tried successfully in skin and vaginal infections due to *Pityrosporum* and *Candida* (1, 5). The side-effect profile is minimal and no consistent deviations from normal haematologic and blood biochemistry values have thus far been observed (1). The efficacy of this drug in the therapy of mycotic keratitis has not hitherto been evaluated. The present study was undertaken, therefore, to determine whether this compound would be useful in the therapy of mycotic keratitis.

**Materials and Methods**

Forty consecutive patients of mycotic keratitis (25 males and 15 females, ranging in age from five years to 60 years) who attended the Cornea Clinic of Joseph Eye Hospital, Trichy, India between July 1, 1985 and November 30, 1985, were treated with itraconazole. These patients (40 eyes) presented with clinical findings of fungal corneal infection and had corneal scrapings performed on the day of admission.

Using a sterile cataract knife, stromal fragments were scraped from the base and margins of each ulcer. These were either placed in a drop of 20% potassium hydroxide to view as a wet mount, or smeared on glass slides for staining by Gram's method. The specimens also were cultured on Brain Heart Infusion agar, MacConkey's agar, Sabouraud's dextrose agar (Emmons' modification) and thioglycollate medium at 35°C for 4 d and on blood agar and Sabouraud's dextrose agar at 20°C for two weeks. The following criteria were adopted for a diagnosis of mycotic keratitis: slit-lamp findings consistent with a diagnosis of mycotic keratitis (11), microscopic observation of hyphal fragments in the potassium hydroxide mount or Gram-stained smear and fungal growth in the C-streaks on at least two culture plates inoculated with material obtained from the lesion.

Prior to the start of therapy, detailed slit-lamp examination was performed and each ulcer was categorised as a severe or a non-severe ulcer, as described previously (10). The ulcer was categorised as non-severe when the ulcer had a diameter of less than 6 mm with ulceration of the superficial one-third and suppuration of the superficial two-thirds of the corneal layers, without either perforation or scleral suppuration. The ulcer was categorised as severe when the ulcer had a diameter of more than 6 mm with ulceration and suppuration involving the deep one-third of the cornea with the presence or possibility of perforation and scleral suppuration.

Itraconazole (R 51211, supplied by Janssen Pharmaceutica, Belgium, Batch Number 85b12/FOI in the form of 50 mg capsules) was initially administered in a dose of 200 mg at one time along with a meal (the starting dose was reduced to 100 mg for two children in the study). If there was an obvious clinical improvement within five days, treatment was continued at the same dosage till the epithelial defect healed; after this, the dose was tapered to 100 mg for five days and then 50 mg for five days. If there was no clinical improvement, or if the ulcer continued to progress, treatment was discontinued and other measures were instituted. The duration of treatment ranged from 8 d to 44 d with 17 d as the median duration. The total dosage administered ranged from 1600 mg to 7000 mg with a median dosage of 3000 mg. Each patient also received systemic analgesics and atropine 1 percent eye drops.

The response of each individual case of keratitis to itraconazole therapy was graded as excellent, moderate or poor depending on the clinical response (as assessed by daily slit-lamp examination) and eradication of the infecting fungus from the lesion (as assessed by repeated mycologic examination). The response was graded as excellent if therapy...
resulted in closure of the epithelial defect, resolution of all lesions and good visual recovery, along with complete eradication of the infecting fungus from the lesion. The response was graded as moderate when itraconazole therapy achieved complete eradication of the fungus from the lesion, but could not by itself achieve complete healing of the ulcer. The response was graded as poor when therapy secured neither complete healing of the lesion nor eradication of the infecting fungus from the lesion.

In vitro susceptibility to itraconazole was determined by an agar dilution method for 18 of the fungous isolates. Kimming agar (E. Merck, Federal Republic of Germany) was used as the test medium since it is recommended for susceptibility testing of the azoles (12). Itraconazole powder (obtained from Janssen Pharmaceutica, Beerse, Belgium) was dissolved in 0.2 N HCl in absolute ethanol. From a stock solution of 2560 μg/ml of itraconazole, the following concentrations of the compound (in μg/ml) in the agar were prepared: 0.063, 0.1, 0.2, 0.4, 0.8, 1, 2, 4, 8, 16, 32, 64, 128. The minimal inhibitory concentrations (MICs) of itraconazole for the 18 fungous isolates were determined by a standard agar dilution method (12). An MIC value of 0.3 μg/ml was chosen to represent a "break point" concentration of itraconazole for the purpose of correlating in vitro susceptibilities of pre-treatment fungous isolates to itraconazole with the results of treatment with itraconazole. This value i.e. 0.3 μg/ml, was chosen since this level of the compound is attained in the serum after oral administration of 200 mg of itraconazole (14). An MIC of 0.3 μg/ml or less for a pre-treatment fungous isolate was interpreted to mean in vitro susceptibility of the isolate to itraconazole and probable excellent or moderate responsiveness of the corresponding clinical case of keratitis to itraconazole therapy. The patients were observed daily for evidence of adverse clinical reactions such as giddiness, nausea, vomiting, headache, gastro-intestinal complaints and pyrosis. The following laboratory investigations were also performed prior to the start of therapy and every two weeks thereafter: routine urinalysis, routine haematological tests and estimation of the levels of total protein, albumin, globulin, fasting glucose, total cholesterol, urea nitrogen and creatinine in the blood.

The Chi-square test and Student's t-test were used to assess the statistical significance of certain results.

Results

The types of fungi isolated from the 40 patients of mycotic keratitis are shown in Table 1.

An excellent response to therapy was observed in 22 patients (14 males and eight females, with a median age of 33 years). This included eight cases of keratitis due to Fusarium solani, nine cases of keratitis due to Aspergillus flavus and one case each of keratitis due to Curvularia geniculata, Drechslera spec., Cladosporium spec. and A. fumigatus; in one case, the causative fungus was not identified. In 15 of these cases, the

<table>
<thead>
<tr>
<th>Fungus</th>
<th>No. of Isolates</th>
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<tbody>
<tr>
<td>Fusarium</td>
<td>19</td>
</tr>
<tr>
<td>F. solani</td>
<td>15</td>
</tr>
<tr>
<td>Other Fusarium spp.</td>
<td>4</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>15</td>
</tr>
<tr>
<td>A. flavus</td>
<td>10</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>5</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>2</td>
</tr>
<tr>
<td>Drechslera spp.</td>
<td>1</td>
</tr>
<tr>
<td>Curvularia geniculata</td>
<td>1</td>
</tr>
<tr>
<td>Botryodiptodia theobromae</td>
<td>1</td>
</tr>
<tr>
<td>Non-sporulating hyaline filamentous fungus (not identified)</td>
<td>1</td>
</tr>
</tbody>
</table>
Fig. 1a: A case of non-severe keratitis due to *Aspergillus flavus* (left eye).
Before treatment, keratitis with 5 mm central ulceration, irregular edges and sloughing, poor visual acuity.

Fig. 1b: A case of non-severe keratitis due to *Aspergillus flavus* (left eye).
Ulcer healed, leaving only a faint corneal scar at the site of the lesion, after 16 days of oral itraconazole therapy (2450 mg); good visual improvement.
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Keratitis was of a non-severe grade while in seven cases, the keratitis was severe. In all 22 cases, progressive corneal ulceration stopped soon after the institution of itraconazole therapy; there was closure of the epithelial defect and complete resolution of all lesions accompanied by good visual recovery. The infecting fungus was completely eradicated from the lesion; specimens taken from the corneal scar after cessation of therapy did not reveal fungi in microscopy or culture. The follow-up period in this group of patients varied from four weeks to 16 weeks; there was no evidence of relapse after cessation of therapy in any of the patients. Figures 1 and 2 show the pre-treatment and post-treatment appearances of two representative cases in this category.

Moderate responsiveness to therapy was observed in five patients (four males and one female with a median age of 40 years). All five patients had keratitis of a severe grade; two cases were due to Fusarium spec., two were due to Aspergillus fumigatus and one was due to Botryodiplodia theobromae. In these patients, the keratitis initially showed improvement but, approximately two weeks after the start of therapy, the ulceration began to progress. Neither bacteria nor fungi were isolated in culture from material taken from the lesion at this time. Penetrating keratoplasty had to be performed in these patients due to excessive corneal thinning and the threat of perforation. Fungi were not isolated in culture from bits of the corneal tissue obtained by surgery.

Poor responsiveness to therapy was observed in 13 patients (seven males and six females with a median age of 40 years). Five of the patients had non-severe keratitis due to Fusarium solani; the other eight patients had keratitis of a severe grade (two due to F. solani, two due to Fusarium spec., two due to Aspergillus fumigatus, one due to A. flavus and one due to Cladosporium spec.). In these 13 patients, progressive corneal ulceration continued in spite of itraconazole therapy, necessitating recourse to penetrating keratoplasty or other measures. The infecting fungi were isolated in culture from material taken from the ulcer during and at the time of stopping therapy.

The minimal inhibitory concentrations (MICs) of itraconazole for the fungal strains isolated from 18 patients were determined as mentioned above. The MICs of itraconazole for these fungi, and the responses to therapy of the patients from which they were isolated, are shown in Table 2. The MICs for five fungal isolates were 0.3 μg/ml or less; excellent responsiveness to therapy was observed in all the five cases from which these fungi were isolated. The MICs for 13 fungal isolates were greater than 0.3 μg/ml; excellent or moderate responsiveness to therapy was observed in 10 of these cases, and a poor response to therapy in three of these cases.

If an MIC value of 0.3 μg/ml was taken to represent a “break point” concentration and based on the interpretation given earlier (see Materials and Methods) then, in eight patients, the in vitro susceptibilities of pretreatment fungous isolates correlated with the results of treatment with itraconazole; that is, in five cases showing excellent responsiveness to therapy, the MICs for the fungous isolates were less than 0.3 μg/ml while in three cases where poor responsiveness to therapy was observed, the MICs for the pretreatment fungous isolates were greater than 0.3 μg/ml. In three out of seven cases of keratitis due to Aspergillus, the excellent response to therapy was in agreement with MIC values of less than 0.3 μg/ml for the fungous isolates. In three out of eight cases of keratitis due to Fusarium, the poor responsiveness to therapy correlated with MICs greater than 0.3 μg/ml for the fungous isolates.

Various factors that possibly influenced the response to therapy with itraconazole were analysed. The type of fungus causing the keratitis appeared to be an important factor influencing the response to therapy. Excellent or moderate responses to therapy were observed in 10 out of 19 (52%) patients with keratitis due to Fusarium, whereas excellent or moderate responsiveness to therapy was observed in 12 out of 15 (80%)
Fig. 2a: A case of severe keratitis due to *Aspergillus flavus* (right eye). Before treatment, keratitis with 7 mm ulceration, extensive sloughing and hypopyon 4 mm; poor visual acuity.

Fig. 2b: A case of severe keratitis due to *Aspergillus flavus* (right eye). Ulcer healed, leaving a dense corneal scar at the site of the lesion, after 25 days of oral itraconazole therapy (3750 mg); moderate visual improvement.
of patients with keratitis due to *Aspergillus*. This difference appeared sizable, although it was not statistically significant ($\chi^2 = 2.762$, 0.1 > p > 0.05).

Other factors analysed were the age and sex of the patients and the severity of the keratitis at the time of commencing therapy. Excellent or moderate responses to therapy were observed in 18 of 25 (72%) male patients and in 9 of 15 (60%) female patients; this sex-related difference was not statistically significant ($\chi^2 = 0.485$, p > 0.5).

The mean age of patients showing excellent or moderate responsiveness to therapy was 34.9 ± 13.5 years while the mean age of patients showing poor responsiveness to therapy was 39.5 ± 13.2 years; this age-related difference was not significant (p > 0.1). Excellent or moderate responses to therapy were observed in 15 of 20 (75%) patients with non-severe keratitis and in 12 of 20 (60%) patients with severe keratitis. This difference was not statistically significant ($\chi^2 = 1.026$, p > 0.30).

Apart from mild gastrointestinal upset in a few patients, there was no evidence of serious adverse reactions to the compound in any of the patients. The haematologic, urine and biochemical parameters analysed were all within normal limits during and after itraconazole therapy.

### Discussion

The therapy of mycotic keratitis is often difficult because the presently available antifungal compounds suffer from various limitations; these limitations include systemic and ocular toxicity (ketoconazole and amphotericin B) (7), poor penetrability into the ocular tissues (pimaricin) (8) and narrow spectrum of antifungal activity (flucytosine) (8). Itraconazole is a new triazole compound which demonstrates antifungal activity after oral administration of the drug; it has proven effective in the therapy of experimental aspergillosis, cryptococcosis and sporotrichosis (16). The efficacy of this compound in the therapy of mycotic keratitis has hitherto not been evaluated and since this condition is an important ophthalmologic problem in India (6), the present study was undertaken.

Excellent responses to oral itraconazole therapy were observed in 55% of the patients treated and moderate responses in
another 12%; in both categories, there was complete elimination of the infecting fungi from the lesions, as demonstrated by mycological investigations. In 33% of the patients, poor responses to itraconazole therapy were observed; the infecting fungi were not eliminated from the lesions in these patients.

Various factors were analysed to determine whether they influenced the responses to treatment with itraconazole. The age and sex of the patients and the severity of the keratitis did not appear to significantly influence the response to treatment. Although a statistically significant correlation could not be derived (perhaps because an insufficient number of cases were studied), the type of fungus causing the keratitis appeared to have some influence on the response to treatment with itraconazole. Thus, in vitro susceptibility data demonstrated that, in vitro, isolates of Aspergillus spp. were relatively susceptible to the antifungal activity of itraconazole whereas the isolates of Fusarium spp. were relatively insensitive to itraconazole as shown by the range of MICs for the isolates. Our in-vitro data for itraconazole and Aspergillus spp. are similar to the data of other workers (4, 16). The relative insusceptibility of ocular isolates of Fusarium spp. to itraconazole is demonstrated for the first time, although similar to the insusceptibility shown by Fusarium spp. for other azoles in-vitro (6). In the clinical setting, excellent or moderate responses to therapy were observed more frequently in cases of keratitis due to Aspergillus than in cases of keratitis due to Fusarium. The responses shown by the cases of Aspergillus keratitis to itraconazole therapy are similar to the results obtained with itraconazole treatment in other clinical conditions where Aspergillus spp. are the aetiologic agents (1, 2).

When a MIC value of 0.3 µg/ml was taken as representing a "break point" concentration with the intention of correlating in-vitro susceptibilities and clinical responsiveness, a positive correlation was observed in 8 of 18 patients. This low degree of correlation between in-vitro studies and clinical responses to antifungal agents has been previously observed (13).

Jones et al. demonstrated the efficacy of clotrimazole and econazole in the therapy of keratitis due to Aspergillus (8, 9). However, both compounds may cause irritation when applied topically; the efficacy of these compounds in the therapy of severe keratitis is also uncertain (3, 9). It remains to be seen whether itraconazole will prove to be more efficient than the older azoles in the therapy of keratitis due to Aspergillus; however, the results of the present study are encouraging. Itraconazole therapy of keratitis due to Fusarium spp. may not prove to be effective, as has been observed with other azoles and Fusarium keratitis (6). We believe that further investigation on this aspect is warranted since 52% of our patients with Fusarium keratitis demonstrated excellent or moderate responsiveness to itraconazole therapy.

This study has demonstrated the potential effectiveness of itraconazole, administrated orally in a dose of 200 mg, as a therapy for mycotic keratitis. In future studies, we propose to evaluate the efficacy of topical and combined oral-topical itraconazole therapy for mycotic keratitis and to determine whether itraconazole is more effective than other compounds as a therapy for keratitis due to Aspergillus.

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References


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