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Itraconazole as most useful drug for fungal corneal ulcers

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Opportunist fungi from exterior environment, which can grow on damaged cornea in an environment of low nutrient and oxygen supply, can casue fungal corneal ulcers. We like many other workers isolated *Aspergillus* spp. from more than half of keratomycosis cases. We also isolated different types of dematiaceous or non-dematiaceous fungi from such lesions. As itraconazole is theoretically preferred drug for most of filamentous fungi, we carried out *in-vitro* study with *Aspergillus flavus* and *Fusarium solani* isolates from such patients for efficacy of available two ophthalmic preparation of antifungal drugs fluconazole and natamycin, along with a prospective drug itraconazole. The latter proved to be superior than other two. Indigenously prepared 1% itraconazole drop, mixing available drug with artificial tears, were tried on such patients by ophthalmologists of different centres and results were encouraging. Subsequently our recommendation came into a reality with "JAWA Pharmaceuticals (India) Pvt. Ltd." prepared the ophthalmic preparation of itraconazole (ITRAL) at the begining of 2002. Human trials of the drug has created a sensation among ophthalmologists.

Key Words : Keratomycosis, antifungal, Itraconazole

INTRODUCTION

Corneal ulcer is a break in the epithelium with underlying stromal necrosis. Fungal corneal ulcers account for large number of cases (Reddy *et al.*, 2000). In most part of the world commonest isolates of corneal ulcers are *Aspergillus* sp. and *Fusarium* sp. (Bennett *et al.*, 1989 ; Maiti *et al.*, 2000) against the fungi isolated from systemic mycosis or keratomycosis and they showed superiority of itraconazole over fluconazole. Fluconzagole and natamycin are at present available two topical ophthalmic preparations against mycotic keratitis. So the scope for use of itraconasole in mycotic keratitis was planned to evaluate by efficacy study of the drug by agar dilution method (Maiti *et al.*, 2000).

On the basis of results obtained from *in-vitro* study, clinical trials were conducted with ophthalmologists of different centres with indegenously prepared ophthalmic preparation in itraconazole suspension. (Agarwal *et al.*, 2001) The results insisted JAWA Pharmaceuticals India Limited to develop first ophthalmic itraconazole preparation with the name of 'ITRAL'. To establish the phamacodynamics and pharmacokinetics of the drug, in the situation, we performed the drug sensitivity test by standard broth dilution method against clinical isolates and reference strains. At the same time the candidate drug (Itral) was tried on selective keratomycotic cases.

MATERIALS AND METHODS

Initially corneal scrapping materials were taken from clinically suspected corneal ulcer patients from different centres and fungus were isolated and identified by standard mycological procedures, then MIC study were determined for the reference stains and with selective clinical isolates.

Clinically suspected cases of fungal corneal ulcers detected during last two years (1999-2001) were selected for study. After performing thorough ocular examinations ophthalmologists reffered these cases for mycological investigations. The scapping materials of the corneal ulcers were done under topical 4% lidocane anaesthesia and clinical materi-

als were inoculated into SDA slants. Smears were prepared with a portion of corneal scapping material, 10% KOH solution and were examined under light microscope for presence of fungal bodies. As the corneal scrapping materials were very often scanty in amount. Gram staining slide examination or bacterial culture were not possible in all cases. The colony morphology and fungal morphology were studied for identification of isolates.

Two reference isolates of *Aspergillus flavus* (MTCC 277) and *Fusarium solani* (MTCC 2671) were taken for drug sensitivity test with commercially available drugs fluconazole 0.3% [Zocon, FDC], natamycin (5%) [Pimafucin. Elder] and itraconazole (1%) Itral, JAWA Pharmaceuticals Ltd. India]. Standard protocol was followed which included the susceptibility testing guide lines described in the NCCLS M 38-P document (NCCLS, 1998). The objectives of this study were to compare durg sensitivity results between different clinical isolates and reference strains.

The set of reference isolates *Aspergillus flavus* MTCC 277, *Fusarium solani* MTCC 2671 belonged to the culture collection of MITC, Chandigarh and 3 clinical isolates of *Aspergillus flavus* and 3 clinical isolates of *Fusarium solani* were included for test.

The liquid medium for this experiment was completely synthetic standard RPM 1640 medium (with glutamine, without bicarbonate and with a pH indicator). This medium was buffered with MOPS (3[N-morpholino] propane-sulfonic acid) at a final concentration of 0.165 mol for pH 7.0 at 25°C.

Three market available ophthalmic preparations of antifungals were used e.g. Zocon. Pimafucin and Itral. All the drug preparations were water base, so their first stock solution were made at 100 times the final concerntration (128 μg/ml) in distilled water followed by further dilution (1:50) in each of the set to yeild two times the final strength required for the test. Microdilution plates (96-U shaped wells) were used for this test. Here in each well drug dilution mixture contained 100 μl drug free media were served as growth control.

Stock inoculum suspensions were prepared as described in the NCCLS M 38-P document (NCCLS, 1998). Conidia formations was induced by growing the isolates on potato dextrose agar at 35°C for 7 days. Seven day old colonies were covered with approximately 1 ml of sterile saline containing 1% Tween 80 and the conidia were harvested by probing the colonies with the tip of pasteur pipette. The resulting mixture of mostly nongerminated conidia were adjusted to optical densities ranged from 0.09 to 0.12 (80-82% transmittance). These suspensions were diluted 1:50 in the media which corresponded to double the density needed for the test which approximately contained 0.4×10^4 to 5×10^4 CFU/ml.

Into each microdilution well 100 μl of the diluted drug was added and inoculated with 100 μl of the diluted conidial suspensions (final volume of drug in each well in 200 μl).

All microdilution trays were incubated at 35°C and examined after 24 hrs. to note end point of growth inhibition.

RESULTS AND DISCUSSION

A total of 88 suspected cases of microbial keratitis were tested from which only fungus was isolated and identified in 35 cases and additional 3 along with the bacterial isolates while only bacteria was isolated in 37 cases and none from 12 cases. Fungus isolates included *Aspergillus* sp. and *Fusarium* sp. in most instances with rare isolates like *Cladosporium, Curvularia, Chaetomium, Pseudoallescheria, Candida* and 5 other unidentified damaticeous fungi.

For several reasons, results of *in vitro* antifungal sensitivity tests for filamentous fungi are not predictive to *in vivo* antimicrobial efficacy. The reproducibility and reliability of the results are often greatly influenced by low solubility of drug in the medium and by composition of medium, pH and inoculum size (Odds *et al.*, 1984). Yet MIC values of drugs of any pre-and post-treatment isolates in identical test conditions can be a good index for evaluation of the therapy and test itself. MIC values of particular drug with different test strains or such values of different drugs against a particular fungus can be compared for prediction of therapeutic use-

fulness. With this aim therapeutic success and failure of a three commonly used topical antifungals were evaluated by following NCCLS M38-P protocol.

Results are shown in Table 1.

Strippoli *et al.* (1988) determined 6.25 to 12.5 µg/ml concentration of itraconazole which completely inhibited spore germination in all test strains of *Aspergillus* sp. conversely in the agar dilution study of Espinel-Ingroff *et al.* (1984) determined MIC_{90} of itraconazole for *Aspergillus* sp. was 0.13 µg/ml. The MIC range of itraconazole was 0.001 to 1 µg/ml for *Aspergillus* sp. and 0.01 to 100 µg/ml for *Fusarium* sp. respectively as determined in brain heart infusion broth by Vancutsem (1989). Maiti *et al.* (2000) showed MIC range of itraconazole tested in Yeast Nitrogen Base Glucose (YNBG) broth following tube dilution method, 0.5 to 2 µg/ml and 4-8 µg/ml for *Aspergillus* sp. and *Fusarium* sp. respectively. In our study MIC ranges were 0.5-3 µg/ml and 1-4 µg/ml for reference strain of *Aspergillus flavus* and for clinical isolates of *Aspergillys flavus* respectively.

Reuben *et al.* (1989) showed MIC range of itraconazole 8-32 µg/ml in 7 test isolates of *Fusarium* sp. Espinel-Ingroff (1998) reported MIC ranges of 1 to > 16 µg/ml for both *F. solani* and *F. oxysporum.* Modal MIC's of > 16 µg/ml were determined for both *Fusarium* spp. in another study by Espinel-Ingroff *et al.* (1997). Another group of workers, Arikan *et al.* (1999) showed no *in vitro* activity of itraconazole (MIC 10 mg/ml) against *F. solani.* For *F. oxysporum* this was 0.15-2 mg/ml. They could not solve out the reasons for their fundings. Muletiadis *et al.* (2000) showed MIC range of itraconazole for *Fusarium solani* as 0.5 to > 32 µg/ml in MIC-O where as the lowest concentration of drug showing slight growth (25%) for *F. oxysporum* was 2→32 µg/ml respectively. Maiti *et al.* (2000) showed MIC range 2 to 8µg/ml in YNBG broth. We found following NCCLS M38-p method 16-64 µg/ml and 16-64 µg/ml in both reference strain set and test isolate set of *F. solani* respectively. Manavathu *et al.* (1996) showed MIC range of fluconazole in 24 clinical isolates of *A. fumigatus* using broth microdilution technique as 128-256 µg/ml which showed that the isolates were resistant to fluconazole. Maiti *et al.* (2000) showed 2-8 µg/ml and 4-8 µg/ml range of MIC values of fluconazole for *Aspergillus* sp. and *Fusarium* spp. Reuben (1989) showed that MIC range of natamycin is 2-4 µg/ml for *Fusarium* sp. Maiti *et al.* (2000) showed that MIC range of natamycin following broth microdilution technique in YNBG solution is 2-16 µg/ml for *Aspergillus* and *Fusarium* respectively. Our study showed the MIC ranges were 64-128µg/ml for *Aspergillus* spp. in both the sets for fluconazole and MIC ranges were 128µg/ml for *Fusarium* sp. in both the sets. Our study showed the MIC ranges of natamycin is 2-8 µg/ml for *Aspergillus* sp. in both the sets and 8-16 µg/ml and 16-32 µg/ml for *Fusarium* sp. in reference set and clinical isolate set respectively.

Espinel-Ingroff (1997) showed that interlab variation of antifungal susceptiability results as high as 50 times, in identical test conditions. So the test methodology is yet to be standardised.

In the context of management of mycotic keratitis concentration of drugs at the target site of avascular cornea not related with the plasma concentration. Available topical preparation of fluconazole contains 3000 µg/ml drug and natamycin contains 50,000 µg/ml. which are so high that theoretically all common keratomycotic agents should be inhibited by direct application of such drugs if retained undiluted and unmodified for a reasonable period of time.

In the treatment of experimental candida-endopthalmitis (Savani *et al.,* 1987) itraconazole was as effective as fluconazole, despite poorer penetration of itraconazole in occular tissues than excellent penetration of fluconazole. The workers evaluated penetration of itraconazole by bioassay in rabbits giving 80mg orally. They observed the itraconazole level in serum and cornea and even in aqueous humor and vitreous body in the inflammed eye. Itraconazole was not detected in the tissues of the uninflammed eye apart from 0.05 µg/ml. in the cornea. So Maiti *et al.* (2000) came to conclusion that following a systemic use, the drug probably concentrates at avascular cornea through tears. This is also previously supported by prospective study of

oral itraconazole in human volunteer trials, where Thomas *et al.* (1988) showed that daily administration of 200 mg itraconazole effected cure in 80% aspergillus keratitis. Cauwenbergh (1988) also showed in human volunteers that achieavable plasma concentration of the drug following a daily 100mg oral dose was 0.34-0.5 μg/ml. Such results indicate that even at much lower concentration than that of topical preparations drugs are quite effective. This information led to shift our attention from pharmacodynamics to pharmacokineties. The results of *in vitro* efficacy study only can serve as a guide line for selection of a drug or drug combinations for clinical trial. Considering the superiority of itraconazole over fluconazole in the treatment of systemic mycosis casued by many similar filamentous fungi prospect for itraconazole in the treatment of mycotic keratitis is justified. From pharmacokinetic point of view itraconazole is expected to be superior drug due to the presence of lipid layer in tears which have the better affinity for lipophilic itraconazole than hydrophilic fluconazole. With this aim we proposed to develop a situable itraconazole preparation for ophthalmic use. Following this scientific basis ophthalmologists started using indigenously prepared 1% itraconazole drop mixing with antificial tears.

During our study this was tried on such patients by ophthalmologists of different centres and results were encouraging. Subsequently our recommendation came to reality when JAWA Pharmaceuticals Pvt. Ltd. (India), prepared the ophthalmic preparation of Itraconazole (ITRAL) at the beginning of 2002. Figures 1 and 2 showed one such patient presented with deep corneal ulcer with complete opacity which was cured on treatment with ITRAL and by direct microscopy and fungal isolation it was proved to be a case of aspergillus keratomycosis.

Table 1 : MIC values of different drugs.

Fungus	MIC values of drugs (μg/ml)		
	Itraconazole	Fluconazole	Natamycin
Reference Strain			
Aspergillus flavus			
MTCC-277	0.5-4	64-128	2-8
Fusarium solani			
MTCC-2671	16-64	128	8-16
Clinical Isolate			
Aspergillus flavus	1-4	64-128	2-8
Fusarium sp.	16-64	128	16-32

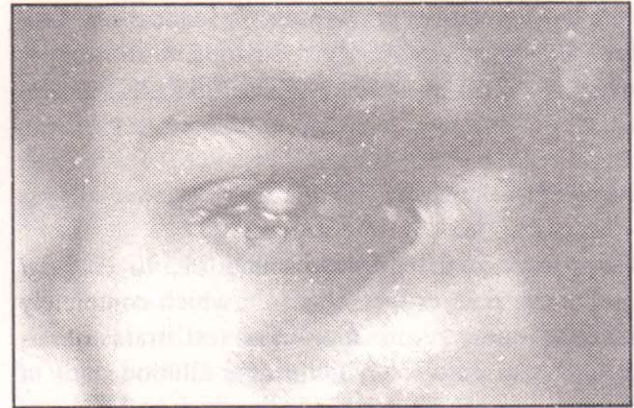

Figure 1. Patient with deep corneal ulcer.

Figure 2. Conidiophore of *Aspergillus fumigatus*.

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