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ORIGINAL ARTICLE/ARTICLE ORIGINAL

In vitro susceptibility of filamentous fungi from mycotic keratitis to azole drugs

Sensibilité in vitro aux azolés de champignons filamenteux, agents de kératite fongique

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KEYWORDS
Mycotic keratitis;
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Summary
Objective. — The in vitro antifungal activities of azole drugs viz., itraconazole, voriconazole, ketoconazole, econazole and clotrimazole were investigated in order to evaluate their efficacy against filamentous fungi isolated from mycotic keratitis.
Methods. — The specimen collection was carried out from fungal keratitis patients attending Aravind eye hospital and Post-graduate institute of ophthalmology, Coimbatore, India and was subsequently processed for the isolation of fungi. The dilutions of antifungal drugs were prepared in RPMI 1640 medium. Minimum inhibitory concentrations (MICs) were determined and MIC_{50} and MIC_{90} were calculated for each drug tested.
Results. — A total of 60 fungal isolates were identified as *Fusarium* spp. ($n = 30$), non-sporulating moulds ($n = 9$), *Aspergillus flavus* ($n = 6$), *Bipolaris* spp. ($n = 6$), *Exserohilum* spp. ($n = 4$), *Curvularia* spp. ($n = 3$), *Alternaria* spp. ($n = 1$) and *Exophiala* spp. ($n = 1$). The MICs of ketoconazole, clotrimazole, voriconazole, econazole and itraconazole for all the fungal isolates ranged between 16 μg/mL and 0.03 μg/mL, 4 μg/mL and 0.015 μg/mL, 8 μg/mL and 0.015 μg/mL, 8 μg/mL

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and 0.015 μg/mL and 32 μg/mL and 0.06 μg/mL respectively. From the MIC_{50} and MIC_{90} values, it could be deciphered that in the present study, clotrimazole was more active against the test isolates at lower concentrations (0.12—5 μg/mL) when compared to other drugs tested.
Conclusion. — The results suggest that amongst the tested azole drugs, clotrimazole followed by voriconazole and econazole had lower MICs against moulds isolated from mycotic keratitis.

MOTS CLÉS
Kératite mycosique ;
Sensibilité aux antifongique ;
Médicaments azolés

Résumé
Objectif. — L'activité antifongique in vitro des azolés à savoir, l'itraconazole, le voriconazole, le kétoconazole, l'éconazole et le clotrimazole a été étudiée afin d'évaluer leur efficacité vis-à-vis des champignons filamenteux isolés de kératite mycosique.
Méthodes. — Les échantillons provenant de patients consultant pour kératite fongique au Aravind Eye Hospital et au Post-Graduate Institute of Ophthalmology, Coimbatore, en Inde ont été mis en culture pour recherche de champignons. Les dilutions des antifongiques ont été réalisées en RPMI 1640. Les concentrations minimales inhibitrices (CMI) ont été déterminées et les CMI_{50} et CMI_{90} ont été calculées pour chaque antifongique étudié.
Résultats. — Soixante souches de champignons ont été isolées: *Fusarium* spp. (*n* = 30), moisissures ne fructifiant pas (*n* = 9), *Aspergillus flavus* (*n* = 6), *Bipolaris* spp. (*n* = 6), *Exserohilum* spp. (*n* = 4), *Curvularia* spp. (*n* = 3), *Alternaria* spp. (*n* = 1) et *Exophiala* spp. (*n* = 1). Les CMI du kétoconazole, du clotrimazole, du voriconazole, de l'itraconazole et de l'éconazole vis-à-vis de l'ensemble des isolats fongiques variaient respectivement entre 16 μg/mL et 0,03 μg/mL, 4 μg/mL et 0,015 μg/mL, 8 μg/mL et 0,015 μg/mL, 8 μg/mL et 0,015 μg/mL et 32 μg/mL et 0,06 μg/mL. À partir des valeurs des CMI_{50} et CMI_{90} que nous avons obtenues, le clotrimazole serait la molécule la plus active vis-à-vis des isolats étudiés, avec des concentrations (0,12 à 5 μg/mL) plus faibles que celles des autres antifongiques testés.
Conclusion. — Les résultats suggèrent que, parmi les antifongiques azolés testés, le clotrimazole suivi par le voriconazole et l'éconazole avaient les CMI les plus basses vis-à-vis des moisissures isolées de kératites mycosiques.

Introduction

Microbial keratitis is the most common severe ocular infection and may be caused by a variety of bacteria, fungi (yeasts, moulds and microsporidia) and protists (e.g. *Acanthamoeba*). It is characterized by an acute or sub-acute onset of pain, conjunctival injection and corneal ulceration with a stromal inflammatory infiltrate [42,16,6,36]. Keratitis due to filamentous fungi is believed to usually occur following trauma, the key-predisposing factor, in healthy young males engaged in agricultural or other outdoor work [8]. The traumatizing agents can be of plant or animal origin (even dust particles), that either directly implant fungal conidia in the corneal stroma, or abrade the epithelium-permitting invasion by exogenous fungi [41]. The etiologic agents of mycotic keratitis show a varying pattern with respect to geographic locality and climatic conditions [7]. More than 105 species of fungi spanning 70 genera have been reported to cause mycotic keratitis [1]. Of these, *Fusarium* spp. and *Aspergillus* spp. are the most common etiological agents of corneal ulcerations [2,5,28].

Pujol et al. [32] reported that amphotericin B (AMB) is probably the most effective drug in vivo, although there have been many clinical treatment failures. Natamycin, a tetraene polyene, has long been considered the mainstay of treatment for filamentous fungal keratitis. Although these drugs have poor ocular penetration, they have primarily been useful in cases with superficial corneal infection [29]. Azoles (imidazoles and triazoles) viz., ketoconazole (KTZ), miconazole (MCZ), fluconazole (FLZ), itraconazole (ITC), econazole (ECN) and clotrimazole (CLT), inhibit fungal ergosterol biosynthesis at low concentrations, while at higher concentrations they appear to cause direct damage to the fungal cell walls [40]. According to Srinivasan [35], ongoing research towards rapid diagnosis and specific drug therapy could minimize the morbidity caused by this preventable disease. The current knowledge on antifungal susceptibilities is mainly based on Western literature and local data available in India pertaining to filamentous fungi other than *Fusarium* and *Aspergillus* are inadequate. The present study was undertaken to isolate and identify filamentous fungi involved in mycotic keratitis from the patients attending a tertiary care eye hospital in Coimbatore, Tamilnadu, India, and to determine their in vitro susceptibility against five azole antifungal drugs by employing the Clinical and laboratory standards institute (CLSI) broth microdilution method M38-A2 document [9].

Materials and methods

Samples and fungal isolates

This non-randomized study was carried out at Aravind eye hospital and Post-graduate institute of ophthalmology, Coimbatore, India. The specimen collection was carried out between October 2012 and August 2013. Corneal scrapings were