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Transparency declarations

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Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


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In vitro activities of antifungal drugs against Rhinocladiella mackenziei, an agent of fatal brain infection

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Sir,

Cerebral phaeohyphomycosis is a rare disease with a mortality of up to 70% despite combinations of surgical and antifungal therapy.1 This infection is predominantly caused by Exophiala dermatitidis, Cladophialaphora bantiana and Rhinocladiella mackenziei (order Chaetothyriales, family Herpotrichiellaceae), although organisms from other orders including Pleosporales, Xylariales and Helotiales can also cause infection. Rhinocladiella is a genus of black yeast-like fungi and can cause human infections ranging from mild cutaneous lesions to fatal brain infections.2 R. mackenziei (formerly Ramichloridium mackenziei) is an extremely neurotropic fungus, and infections that invade the CNS cause death in most instances.1,2 The infection is restricted largely to the Middle East, especially Saudi Arabia and Kuwait, although sporadic cases involving visitors or immigrants from endemic areas have been diagnosed in the UK and the USA. No clear epidemiological factors other than area of residence link these patients. Most R. mackenziei infections are brain abscesses in patients with no predisposing factors or immunodeficiency.1,2 Symptoms included headache, neurological deficits and seizures. Pathogenesis may be haematogenous from a subclinical pulmonary focus, although it remains unclear why this fungus preferentially causes CNS disease in immunocompetent individuals.2 Treatment regimens, which involve mostly amphotericin B, itraconazole and 5-flucytosine, are associated with a poor outcome in both animal and clinical studies.3 R. mackenziei demonstrates in vitro resistance to amphotericin B but susceptibility to triazoles such as itraconazole.3–5 Experimental R. mackenziei cerebral infection in mice indicates that posaconazole is superior to either amphotericin B or itraconazole for treatment of this condition.3

Here we describe the in vitro activity of a new triazole antymycotic, isavuconazole, and seven comparators against 10 clinical isolates of R. mackenziei. Strains of R. mackenziei (nine from cerebral abscesses, one from a liver abscess) were obtained from the CBS-KNAW Fungal Biodiversity Centre (Utrecht, The Netherlands) and identified as R. mackenziei by sequencing of the internal transcribed spacer regions of rDNA. Antifungal susceptibility testing was performed in microdilution plates according to CLSI guidelines after 96 h of incubation at 35°C. Amphotericin B (Bristol–Myers Squibb, Woerden, The Netherlands), fluconazole (Pfizer Central
Research, Sandwich, Kent, UK), voriconazole (Pfizer), anidulafungin (Pfizer), itraconazole (Janssen Research Foundation, Beerse, Belgium), posaconazole (Schering-Plough, Kenilworth, NJ, USA), isavuconazole (Basilea Pharmaceutica International Ltd, Basel, Switzerland) and caspofungin (Merck Sharp & Dohme BV, Haarlem, The Netherlands) were obtained as reagent grade powders. The antifungal agents were dispensed into microdilution trays at a final concentration of 0.016–16 mg/L for amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin, 0.063–64 mg/L for fluconazole and 0.008–8 mg/L for isavuconazole and anidulafungin. Conidial inocula were prepared under biosafety laboratory (level 3) regulations from 2-week potato dextrose agar cultures by gently scraping the surface of mature colonies with a sterile cotton swab moistened with sterile physiological saline containing Tween 40 (0.05%). Supernatants were adjusted spectrophotometrically (530 nm) to a percentage transmission in the range 68–71 (corresponding to 1.5–4 \( \times 10^6 \) cfu/mL). Quality control strains Paecilomyces variotii ATCC 22319, Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were included in each assay run.

The MIC endpoint for amphotericin B, itraconazole, voriconazole, posaconazole and isavuconazole, determined with the aid of a magnifying mirror, corresponded to the lowest drug concentration resulting in wells without any recognizable growth; the MIC endpoint for fluconazole was defined as the minimum drug concentration resulting in a prominent reduction (\( \geq 50\% \)) of growth. For the echinocandins, MICs were determined microscopically as the lowest concentration of caspofungin or anidulafungin leading to formation of small, rounded, compact hyphal forms rather than the long unbranched hyphal clusters seen in growth controls devoid of antimycotic.

The origin, CBS identification numbers, clinical data and MIC values for the \( R. \) mackenziei isolates surveyed are summarized in Table 1. MIC ranges for amphotericin B (2–16 mg/L), voriconazole (0.25–2 mg/L) and anidulafungin (1–8 mg/L) ranged over 3 log₂ dilution steps, whereas MIC ranges for the remaining azoles ranged over 2 log₂ dilution steps, and the MIC range for caspofungin (4–8 mg/L) ranged over 1 log₂ dilution step. Fluconazole had high MICs, indicating poor activity against this pathogen, while the lowest MIC values were seen for posaconazole (MIC₉₀ 0.063 mg/L). Drug treatment of human \( R. \) mackenziei cerebral phaeohyphomycosis, according to several case reports, has been unsuccessful despite aggressive therapy with amphotericin B and/or itraconazole, with or without 5-flucytosine and irrespective of surgical intervention; only a single patient is reported to have survived an \( R. \) mackenziei cerebral infection, with pronounced radiological and clinical improvement after switching therapy from a combination of liposomal amphotericin B and itraconazole to posaconazole. This sole surviving patient relapsed on itraconazole and displayed symptoms of progressive disease after several months of treatment with voriconazole, and the MIC for posaconazole remained below 0.5 mg/L, indicating poor activity. The MIC for caspofungin increased to 1 mg/L, corresponding to drug concentrations of 0.125 mg/L, 1 mg/L and 4 mg/L for amphotericin B, itraconazole and posaconazole, respectively. The MIC endpoint for posaconazole, determined by microscopic examination of the mycelium, corresponded to the lowest drug concentration resulting in visible growth of a morphologically distinct, compact hyphal form morphology, as compared to the long unbranched hyphal clusters seen in growth controls devoid of antimycotic. The MIC endpoint for amphotericin B, itraconazole and posaconazole, determined by microscopic examination of the mycelium, corresponded to the lowest drug concentration resulting in visible growth of a morphologically distinct, compact hyphal form morphology, as compared to the long unbranched hyphal clusters seen in growth controls devoid of antimycotic.
supports use of posaconazole for this difficult to treat infection. Most melanized fungi appear to be non-susceptible to echinocandins, probably due to a reduced presence of β-glucan in their cell walls, and we found poor activity of caspofungin and anidulafungin against R. mackenziei (MIC<sub>90</sub> 8 mg/L). Posaconazole and itraconazole (MIC<sub>90</sub> 0.25 mg/L) demonstrated the best in vitro activities, followed by isavuconazole (MIC<sub>90</sub> 1 mg/L), though clinical experience strongly suggests that itraconazole is not a suitable choice for treating an R. mackenziei cerebral infection. The new drug isavuconazole may be a promising alternative for this indication, but its activity against R. mackenziei needs to be confirmed in animal models.

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