Scopulariopsis brevicaulis, a Fungal Pathogen Resistant to Broad-Spectrum Antifungal Agents

Manuel Cuenca-Estrella,* Alicia Gomez-Lopez, Emilia Mellado, Maria J. Buitrago, Araceli Monzón, and Juan L. Rodríguez-Tudela

Unidad de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid, Spain

Received 21 January 2003/Returned for modification 3 March 2003/Accepted 15 April 2003

The antifungal susceptibility results for 32 clinical isolates of Scopulariopsis brevicaulis are presented. Fluconazole and itraconazole were inactive in vitro, and MICs of amphotericin B, voriconazole, and terbinafine for all isolates were high, with geometric means of 13, 25.8, and 14.4 μg/ml, respectively.

Scopulariopsis spp. are common soil saprophytes and have been isolated from a wide variety of substrates (10). They are cosmopolitan organisms, and five species have been associated with human diseases: Scopulariopsis brevicaulis, Scopulariopsis brumptii, Scopulariopsis acremonium, Scopulariopsis fusca, and Scopulariopsis koningii. They are dermatomycotic molds and mainly have been associated with onychomycosis (19, 20).

S. brevicaulis rarely has been reported as a cause of deep fungal infections, but in the last 2 decades a number of severe illnesses have been documented in hosts presenting factors which predispose them to infection. The spectrum of severe human mycoses includes the formation of fungus balls in preformed pulmonary cavities (6), keratitis (11), posttraumatic endophthalmitis (7), disseminated skin lesions in AIDS patients (5), granulomatous subcutaneous infections (3), invasive hyalohyphomycosis (17, 18), pneumonia in leukemic patients (21), endocarditis related to valvuloplasty or prosthetic valves (8, 12, 14), and fatal disseminated infection after bone marrow transplantation (13, 16). In addition some isolates of S. brevicaulis have been demonstrated to be pathogenic in murine models of disseminated infection (10).

Optimal treatment of these fungal infections is unknown. Debridement or excision of necrotic tissue and antifungal chemotherapy should be the treatments of choice (18). But indications for surgery are limited, and the dose and duration of chemotherapy are not established. Prognosis depends mainly on the patient’s immune status and feasibility of surgical debridement (13). Moreover, several authors have suggested that S. brevicaulis is resistant in vitro to amphotericin B, fluconazole, and azole compounds (2, 9).

This study describes the susceptibility in vitro to broad-spectrum antifungal agents of clinical isolates of S. brevicaulis.

(A work was presented in part at the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, Calif., 2002.)

Fungi. A collection of 32 clinical isolates was included. All strains were recovered during a period of 3 years (2000 to 2002) from 17 Spanish hospitals. Each clinical isolate represented a unique isolate from a patient. Paecilomyces variotii (ATCC 22319) and Aspergillus fumigatus (ATCC 9197) were used as reference strains to control the quality and to monitor the reproducibility of susceptibility tests. The isolates were sent to the Mycology Unit for antifungal susceptibility testing with a referral note including both clinical and microbiological data. All strains were isolated from nail or skin scrapings. A total of 25 of 32 (78.1%) isolates were associated with onychomycosis. There was no history of antifungal treatment for 27 of 32 (84.4%) patients.

All isolates grew on cycloheximide-containing medium and formed spreading colonies on 2% malt extract agar. Colonies had a powdery-to-felty surface and were greyish white at first, later becoming pinkish brown. The reverse was cream colored to brownish. Microscopic examination revealed lemon-shaped, roughened conidia with truncated bases produced from the tips of annellidic conidiogenous cells. The annellides were produced singly or in penicillate heads.

Antifungal agents. Antifungal agents utilized were amphotericin B (Sigma-Aldrich Química, Madrid, Spain), fluconazole (Sigma-Aldrich Química), terbinafine (Novartis Pharma AG, Basel, Switzerland), itraconazole (Janssen Pharmaceutical, Madrid, Spain), and voriconazole (Pfizer Ltd., Sandwich, United Kingdom). They were obtained as standard powders, and stock solutions were prepared in 100% dimethyl sulfoxide (Sigma-Aldrich Química), except for fluconazole, which was dissolved in sterile distilled water.

Antifungal susceptibility testing. A broth microdilution test was performed in accordance with the NCCLS reference method (15), with minor modifications. The susceptibility testing medium was RPMI 1640 with 1-glutamine buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid (MOPS)—10 M NaOH (Oxoid, Madrid, Spain) and supplemented with 18 g of glucose per liter (RPMI 1640—2% glucose). This medium was prepared as a double-strength solution. Inoculum suspensions were prepared from fresh, mature (3- to 5-day-old) cultures in accordance with a methodology reported previously (1). Briefly, the colonies were covered with 5 ml of distilled sterile water containing 1% Tween 20 (Sigma-Aldrich Química). Then, the conidia were carefully rubbed with a sterile cotton swab (Collection swab; EUROTUBO, Madrid, Spain).
and transferred to a sterile tube; the resulting suspensions were homogenized for 15 s with a gyratory vortex mixer at 2,000 rpm (MS 1 Minishaker; IFA, Cultek, Madrid, Spain). The inoculum size was adjusted to a range of 1.0 × 10⁷ to 5 × 10⁸ spores/ml by microscopic enumeration with a cell counting hemocytometer (Neubauer chamber; Merck, S.A., Madrid, Spain). All adjusted suspensions were quantified by plating on Sabouraud agar plates.

Sterile plastic microtitration plates with 96 flat-bottom wells each were employed. These plates contained twofold serial dilutions of the antifungal drugs and two-drug-free medium wells for sterility and growth controls. The inoculum suspension was then diluted 1:10 with sterile water to get a final working inoculum of 1 × 10⁵ to 5 × 10⁵ CFU/ml. This inoculum size is 10-fold higher than that recommended by the NCCLS M38-A protocol. However, some reports have demonstrated that inoculum sizes of 1 × 10⁵ to 5 × 10⁵ CFU/ml generate reproducible in vitro susceptibility data for Aspergillus spp. that can predict clinical outcome. In addition the higher inoculum size does not have a significant influence on MICs (4).

The trays were inoculated with 0.100 ml in each well and were incubated at 35°C for 24, 48, and 72 h in a humid atmosphere. Visual readings were performed with the help of a mirror.

**End point determination.** MICs were defined as the lowest concentrations of the antifungal agents that completely inhibited fungal growth.

**Statistical analysis.** Statistical analysis was done with Statistical Package for the Social Sciences (version 11.0; SPSS S.L., Madrid, Spain).

Table 1 displays geometric means, modes, and ranges of MICs for the 32 isolates tested. All fungi produced detectable growth after 48 to 72 h of incubation. Fluconazole and itraconazole were inactive in vitro against all isolates. Amphotericin B, voriconazole, and terbinafine exhibited slightly better activity in vitro than fluconazole and itraconazole. For five strains (15.6%), the amphotericin B MIC was 4 µg/ml, and for seven isolates it was 8 µg/ml. The MICs of the five agents for the control organisms were consistent within two or three twofold dilutions. These values are displayed in Table 2.

*S. brevicaulis* is an annellidic hyphomycete belonging to the division Ascomycota. The information relating to the susceptibility of this species to antifungal agents is sparse and somewhat contradictory. However, it should be emphasized that interpretative breakpoints for susceptibility testing of filamentous fungi are not available and clinical studies with this organism have not been reported. Aguilar et al. reported the susceptibilities of five strains of *S. brevicaulis* (2). Isolates were resistant in vitro to amphotericin B, fluconazole, flucytosine, itraconazole, miconazole. On the other hand, MICs of ketoconazole were lower, averaging 1 µg/ml. In addition, Johnson et al. reported the resistance in vitro to amphotericin B and itraconazole of five strains of *S. brevicaulis* (9). The studies of Aguilar et al. and Johnson et al. were performed in accordance with the NCCLS method for filamentous fungi. However, a study by Wildfeuer et al., using a different susceptibility testing procedure, reported lower average MICs of amphotericin B, itraconazole, ketoconazole, and voriconazole for 22 isolates: 2.59, 1.47, 0.71, and 1.7 µg/ml, respectively (22).

Herein we present the antifungal susceptibility results for 32 clinical isolates of *S. brevicaulis*. The results show that MICs of all tested antifungal compounds for *S. brevicaulis* isolates are very high. It can be concluded that this hyphomycete species is multiresistant to broad-spectrum antifungal agents available today. It could be intrinsically resistant to antifungal agents since 84.4% of our cases had no history of antifungal treatment. Because of its resistance, invasive infections due to *S. brevicaulis* are unlikely to respond to particular antifungal treatment and other therapeutic approaches should be considered (e.g., combined therapy and immunotherapy), particularly in immunosuppressed patients with disseminated mycoses.

A. Gómez-López is Fellow of the Fondo de Investigaciones Sanitarias (grant 99/198).

**REFERENCES**


