Activities of D0870, a Novel Triazole, against Candida lusitaniae and Trichosporon beigelii in Experimental Murine Infections

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Candida lusitaniae and Trichosporon beigelii may cause life-threatening infections in the immunocompromised host and may be resistant to amphotericin B. We assessed the activities of a new triazole, D0870, against one T. beigelii and four C. lusitaniae strains, in comparison with those of fluconazole and amphotericin B. Immunosuppressed CF1 mice, intravenously infected with each fungal strain, received 3 days of therapy with oral D0870 (5 or 25 mg/kg of body weight daily), fluconazole (5 to 50 mg/kg daily), or parenteral amphotericin B (1 or 2 mg/kg daily). Survival was significantly prolonged and kidney fungus titers were reduced in mice treated with D0870 compared with untreated mice (P \leq 0.05). Treatment with D0870 was significantly more effective than that with amphotericin B or fluconazole in animals infected with two of the C. lusitaniae strains and equally effective for the remaining two C. lusitaniae strains and the T. beigelii strain. Fluconazole and amphotericin B failed to improve the survival of mice infected with one and two C. lusitaniae strains, respectively. D0870 was active against all the organisms tested, including those resistant to fluconazole and amphotericin B.

Candida lusitaniae and Trichosporon beigelii can cause life-threatening hematogenous infections in patients with hematology malignancies (1, 5, 13, 14). Both agents are frequently resistant to amphotericin B, and management of infections caused by these organisms may be difficult (16, 22).

We tested the in vitro and in vivo antifungal activities of D0870, a new orally administered bis-triazole, in comparison with those of fluconazole and amphotericin B against one T. beigelii strain and four C. lusitaniae strains. D0870 is the mycosologically active R-enantiomer of ICI195,739, which shows promise as a potent broad-spectrum antifungal agent (7–12, 17–21, 23) and in a recent clinical pilot study proved safe and effective for the treatment for AIDS patients with oropharyngeal candidiasis unresponsive to fluconazole (6).

Animals. Four-week-old CF1 male mice (average weight, 25 g; Harlan-Sprague Breeding Laboratories, Indianapolis, Ind.) were used. Animal studies were performed in accordance with the guidelines of the Animal Welfare Act and the National Institutes of Health for the care and use of laboratory animals.

Organisms. One C. lusitaniae strain (CL 5W31) known to be resistant to amphotericin B and one T. beigelii isolate (TCM 756) were kindly provided by W. G. Merz (Johns Hopkins Medical Institutions, Baltimore, Md.) and M. G. Rinaldi (The University of Texas Health Science Center, San Antonio, Tex.), respectively. Three additional C. lusitaniae isolates (CL 1706, CL 2819, and CL 524) were recovered from the blood of patients cared for at The University of Texas M. D. Anderson Cancer Center, Houston, Tex. All isolates were maintained in water stock and subcultured on Sabouraud’s dextrose agar (SDA) plates. Inocula of C. lusitaniae for in vitro and in vivo studies were prepared by suspending yeast cells in sterile 0.9% NaCl and adjusting the mixture to 5% spectrophotometric transmission (Spectronic 20; Bausch & Lomb, Rochester, N.Y.). Inocula for the T. beigelii experiments were prepared as follows. Twenty-four hours after incubation at 35°C, 5 ml of 0.9% NaCl was added to the SDA plates. A bent glass rod was gently used to dislodge the colonies of T. beigelii from the plate medium. The suspension was then filtered to remove hyphae and remaining clusters of organisms. Sterile 0.9% NaCl was added as needed to obtain 40% transmittance by spectrophotometry. Hemacytometry counts confirmed the presence of <10% hyphal forms of T. beigelii in the final suspension. Colony counts for C. lusitaniae and T. beigelii inocula were verified by serial dilution on SDA plates.

Antifungal agents. Fluconazole was received as a powder from Pfizer Inc. (Groton, Conn.). Amphotericin B was purchased as Fungizone from E. R. Squibb & Sons (Princeton, N.J.). Micronized D0870 was provided by Zeneca Pharmaceutical Corp. (Macclesfield, United Kingdom). All drugs were prepared and maintained in solution according to the manufacturers’ recommendations. For in vitro testing, fluconazole, amphotericin B, and D0870 were dissolved in dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Mo.) and diluted with RPMI 1640 (Sigma Chemical Co.) to the highest concentrations of 64 \mu g/ml for fluconazole and D0870 and 10 \mu g/ml for amphotericin B.

Susceptibility testing. In vitro susceptibility tests were performed with a previously described broth microtiter method with agitation (3). A strain of Candida albicans (ATCC 64550) served as a control organism. The MIC end-point was defined as a \geq 50% reduction in growth. Reading was done at 24 h.

In vivo experiments. Mice selected for inoculation with C. lusitaniae received 5-fluorouracil (125 mg/kg of body weight intraperitoneally 24 h before challenge) and cortisone acetate (125 mg/kg subcutaneously 24 h before the inoculation and 75 mg/kg subcutaneously daily for the next 2 days), whereas those selected for inoculation with T. beigelii received cyclophosphamide (150 mg/kg intraperitoneally 24 h before challenge). Groups of 20 mice were injected intravenously with the infecting inoculum containing 5 \times 10^7 \pm 3 \times 10^7 CFU of each of the
C. lusitaniae strains or 5 × 10^6 conidia of the T. beigelii strain. Therapy was initiated 1 h after inoculation and continued for 3 consecutive days. Mice infected with C. lusitaniae were randomized to receive 1 mg of amphotericin B per kg intraperitoneally or two doses of the triazoles: 5 or 25 mg of D0870 or fluconazole per kg orally.

Twenty-four hours after the end of treatment, five randomly selected mice from each experimental group were killed, and their kidneys were removed, weighed, and homogenized with 5 ml of sterile 0.9% NaCl in a Stomacher blender (Seward Medical, London, United Kingdom). Samples from each specimen were serially diluted and plated on SDA plates, and the number of CFU per gram of tissue was calculated. Survival of the remaining animals was monitored for up to 21 days. All experiments were repeated to confirm reproducibility.

**Statistical analysis.** Survival and organ clearance data for each group of treated mice were compared with those from untreated mice by using the Mann-Whitney U test; significance was defined as P of ≤0.05.

**In vitro antifungal activities.** MICs of amphotericin B, fluconazole, and D0870 against the strains tested are shown in Table 1. D0870 was active in vitro against all five isolates. The MICs of amphotericin B and fluconazole were one- to fourfold higher than that of D0870.

**In vivo antifungal activities.** Survival of mice infected with each C. lusitaniae or T. beigelii strain was significantly prolonged by D0870 treatment, with overall survival ranging from 60 to 100%, compared with <25% for untreated mice (P < 0.05) (Table 2). The mean number of CFU per gram of kidney tissue was 1.5 to 3 orders of magnitude lower in the D0870 groups than in controls (P ≤ 0.05). Results for survival and organ clearance of fungi in mice infected with C. lusitaniae and T. beigelii are shown in Tables 2 and 3, respectively. Amphotericin B significantly prolonged the survival of mice infected with two of the four C. lusitaniae strains (CL 1706 and CL 524) and the T. beigelii strain (P ≤ 0.05 versus control) but not of mice infected with the other two C. lusitaniae strains (CL 2819 and CL 5W31 [Table 2]). Amphotericin B lowered kidney fungus titers in mice infected with each strain tested (P ≤ 0.05 versus control). This effect, however, was less prominent in mice infected with CL 2819, CL 5W31, and the T. beigelii strain (<1 order of magnitude reduction in the mean number of CFU per gram of tissue compared with that for controls [Table 3]). Fluconazole treatment significantly improved survival of and lowered kidney fungus titers in mice infected with all (P ≤ 0.05 versus control) but one strain (CL 524). D0870 was more active than amphotericin B against infection caused by CL 2819 (P < 0.01) and CL 5W31 (P < 0.001) and was equally active against infection caused by the remaining C. lusitaniae strains. D0870 was superior to fluconazole for the treatment of mice infected with CL 524 (P < 0.01) and was comparable to fluconazole for the remaining C. lusitaniae strains. There was no statistically significant difference between the doses (5 or 25 mg/kg) of D0870 tested in mice with disseminated trichosporonosis, although a dose-dependent effect in the reduction of tissue burden was observed (Table 3). D0870 given at 5 mg/kg was as effective as 25 mg/kg and significantly more effective than an equal dose of fluconazole (P < 0.05) in increasing the survival rate. At both doses, D0870 was significantly (P < 0.005) better than amphotericin B in lowering T. beigelii titers in the kidneys of infected mice.
Our results demonstrate that D0870 is active in vitro and in vivo against C. lusitaniae and T. beigeli. Amphotericin B and fluconazole also showed high degrees of activity against most of the strains tested. D0870 improved the outcome of immunosuppressed mice infected with clinical isolates of C. lusitaniae that did not respond to amphotericin B or fluconazole therapy. However, none of the antifungal agents tested could cure the infections under the experimental conditions used. In our experiments we tested up to 25 mg of D0870 per kg daily, because preliminary studies in our laboratory (2) and others (7, 8) showed that higher dosages are associated with early mortality attributable to drug toxicity. Compared with D0870, fluconazole was five times less active against trichosporonosis (Table 3) and had to be administered twice daily to produce results comparable to those with an equal dose of D0870 given once daily. This finding could be explained by the longer half-life (24 h) of D0870 in mice (7), compared with that of fluconazole. Preliminary experiments in our laboratory showed that fluconazole given twice daily was significantly more active against C. lusitaniae than an equal dose given once daily (2).

Our data, although very encouraging for the efficacy of D0870, are derived from experiments testing a small number of fungal isolates. Nevertheless, our study is in agreement with recent favorable reports on D0870 against various fungi. D0870 has a broad spectrum of in vitro antifungal activity against Candida species (12, 19, 23), Cryptococcus neoformans (12, 19, 23), Blastomyces dermatitidis (7, 20), Aspergillus fumigatus (18, 20, 23), and other molds (20). The MICs of D0870 against Candida krusei (19) and Torulopsis glabrata (4, 20) were higher than those against various Candida species. Several models of experimental murine infections were used for the in vivo testing of D0870. Vaginal (11, 17) and disseminated (11, 12, 23) candidiasis caused by C. albicans, disseminated (12, 23) and pulmonary (17) cryptococcosis, cryptococcal meningitis (9), pulmonary blastomycosis (7), hematogenous aspergillosis (23), and coccidioidomycosis (8) in normal and immunosuppressed mice responded more favorably to D0870 than to fluconazole. The activity of D0870 in experimental candidiasis caused by Candida species other than C. albicans was variable. D0870 was active in vitro and in vivo against only one of four of T. glabrata strains tested (4) and was not more effective than fluconazole in hematogenous infection caused by a C. krusei isolate (15). Additional experiments with a different strain of C. krusei confirmed the limited efficacy of the investigational triazole against this species (2). Our present work is the first to demonstrate significant activity of D0870 in immunosuppressed mice hematogenously infected with C. lusitaniae strains that were resistant to fluconazole and amphotericin B. In conclusion, D0870 has considerable in vitro and in vivo antifungal activity against C. lusitaniae and T. beigeli. This novel triazole holds promise as a therapeutic alternative against opportunistic mycoses refractory to currently available antifungal agents and deserves further testing.

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