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Activity of Voriconazole (UK-109,496) against Clinical Isolates of *Aspergillus* Species and Its Effectiveness in an Experimental Model of Invasive Pulmonary Aspergillosis

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Voriconazole, a new azole antifungal agent, showed potent activity against clinical isolates of *Aspergillus* spp. in vitro. For *A. fumigatus*, the MIC range was <0.03 to 0.5 μg/ml and the MIC at which 90% of isolates are inhibited was 0.25 μg/ml. In an experimental model of invasive pulmonary aspergillosis which mimics infection in humans, oral voriconazole at dosages of 30 mg/kg of body weight per day significantly delayed or prevented mortality.

Invasive aspergillosis is an important cause of morbidity and mortality in the immunocompromised host, particularly in patients with acute leukemia and bone marrow transplant recipients (4, 5, 8, 11, 13, 19, 20, 24). Despite variable efficacy and considerable toxicity, amphotericin B remains the treatment of choice for this condition (5, 8). Itraconazole, a triazole antifungal agent, is active against *Aspergillus* spp. in vitro and has been used successfully to treat invasive aspergillosis in immunocompromised patients (6, 14). However, the lack of an intravenous preparation and variable absorption following oral administration in patients receiving chemotherapy or bone marrow transplant recipients limits the utility of itraconazole in many patients. Low levels of the drug in serum have been associated with therapeutic failure and poor outcome (6, 14).

Voriconazole (UK-109,496) is a potent new triazole derivative with a broad spectrum of antifungal activity against many opportunistic fungal pathogens including *Candida*, *Cryptococcus*, and *Aspergillus* species (1, 2, 15). In an experimental model of disseminated aspergillosis, oral voriconazole prevented mortality in infected animals (12). In this study, we evaluated the in vitro activity of voriconazole against a range of clinical isolates of *Aspergillus* spp. and its efficacy in a rat model of invasive pulmonary aspergillosis (IPA).

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For antifungal susceptibility testing we used a modification of the microdilution method that has been proposed by the National Committee for Clinical Laboratory Standards as the standard reference method for yeasts (10). Clinical isolates from the Memorial Hospital Microbiology Laboratory were subcultured on Sabouraud dextrose agar and were incubated at 30°C for 3 to 4 days. Conidia were harvested with a 0.02% Tween 80 solution, centrifuged (10 min, 1,500 × *g*), suspended in distilled water, and counted in a hemacytometer. Conidia were diluted with RPMI medium (American Biorganics, Inc., Niagara Falls, N.Y.) to obtain approximately 2×10^4 CFU/ml or two times the final test inoculum. Stock drug solutions of voriconazole (5 mg/ml; Pfizer-Limited, Sandwich, United Kingdom), itraconazole (1 mg/ml; Janssen Pharmaceutica Inc., Piscataway, N.J.), and amphotericin B (5 mg/ml; Bristol-Myers Squibb, Princeton, N.J.) were prepared in dimethyl sulfoxide. Stock solutions were diluted with RPMI medium to obtain twofold strengths of the final test concentrations (64 μg/ml) for the broth microdilution assay. The broth microdilution tests were performed with sterile, disposable, multiwell microdilution plates (96-well U-shaped Nunclon MicroWell Plate; Fisher Scientific, Pittsburgh, Pa.). Twofold serial dilutions of test drug concentrations were made. Portions of conidial suspensions (100 μl) were combined with an equal volume of drug solution to give a final inoculum concentration of 10^4 conidia/ml. The plates were incubated at 35°C for 48 h. The lowest concentration of drug that prevented visible growth was considered the MIC.

All animal research procedures were approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Willmington, Mass.) weighing 125 to 150 g received cortisone acetate (100 mg/kg of body weight) subcutaneously thrice weekly, tetracycline via drinking water, and a low-protein (8%) diet (Dyets Inc., Bethlehem, Pa.) throughout the course of the trials. Following the admin-

TABLE 1. Results of tests of in vitro susceptibilities of *Aspergillus* spp. to voriconazole, itraconazole, and amphotericin B

Drug	MIC (μg/ml)[a]								
	A. fumigatus (*n* = 21)			*A. flavus* (*n* = 10)			*A. niger* (*n* = 10)		
	Range	90%	50%	Range	90%	50%	Range	90%	50%
Voriconazole	<0.03–0.5	0.25	0.03	0.25–0.5	0.5	0.5	0.25–1.0	0.5	0.25
Itraconazole	<0.03–1.0	0.5	0.06	0.125–0.25	0.25	0.25	0.5–2.0	1.0	1.0
Amphotericin B	0.5–2.0	2.0	1.0	1.0–4.0	4.0	2.0	0.5–1.0	1.0	0.5

[a] 90% and 50%, MICs at which 90 and 50% of isolates, respectively, are inhibited.

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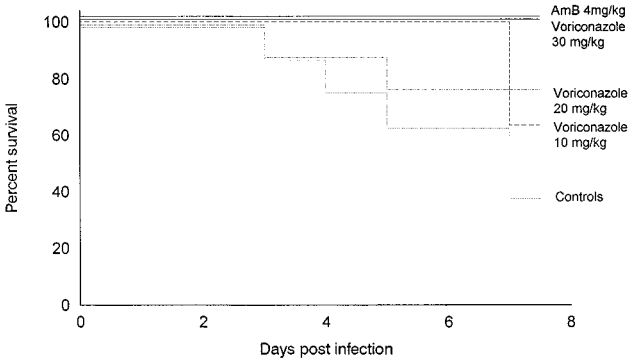

FIG. 1. Kaplan-Meier plots showing survival of rats with pulmonary aspergillosis after treatment with placebo, amphotericin B (AmB; given intraperitoneally), or escalating oral doses of voriconazole.

istration of a single oral dose (30 mg/kg) of voriconazole or itraconazole, blood samples were obtained at timed intervals over 8 h. The levels of voriconazole and itraconazole in serum were determined by modifications of a previously reported agar diffusion bioassay incorporating *Candida kefy*r (MSK strain; CPS-2) (3, 16, 18). The concentrations of the test samples in serum were calculated by an equation, derived by linear regression, relating the diameters of zones of inhibition to the log of the drug concentrations in the standards. After 2 weeks of this immunosuppressive regimen, groups of rats were infected via the trachea with a suspension of 10^6 conidia of *A. fumigatus* H11-20 in 0.1 ml of sterile saline under enflurane (Ethrane; Anaquest) anesthesia. In trial 1, beginning at the time of infection, groups of rats ($n = 8$ rats per group) were treated once daily for 5 days with oral doses of 10, 20, or 30 mg of voriconazole per kg suspended in corn oil, an equal volume of corn oil (controls), or intraperitoneal doses of amphotericin B (4 mg/kg in 5% dextrose water). In trial 2, the effectiveness of voriconazole and itraconazole was compared at doses of 30 mg/kg administered by gavage ($n = 12$ per group); doses (in corn oil) were given at the time of infection and then daily for 5 days. Outcome was determined by survival analysis by using Kaplan-Meier plots and the log rank test. Histological examination of the lungs from representative samples of each group was made.

Table 1 presents the MICs of voriconazole, itraconazole, and amphotericin B. Voriconazole had lower MICs than amphotericin B for all clinical isolates of *Aspergillus* spp. Voriconazole was more active than itraconazole against strains of *A. fumigatus* and *A. niger* and was slightly less active against strains of *A. flavus*. The MICs of amphotericin B, itraconazole, and voriconazole for the H11-20 strain of *A. fumigatus* used in the animal experiments were 0.25, 0.125, and 0.25 μg/ml, respectively.

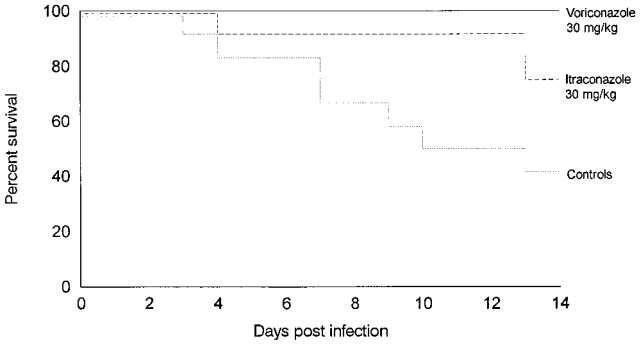

FIG. 2. Kaplan-Meier plots showing survival of rats with pulmonary aspergillosis after treatment with placebo, voriconazole, or itraconazole (30 mg/kg given orally).

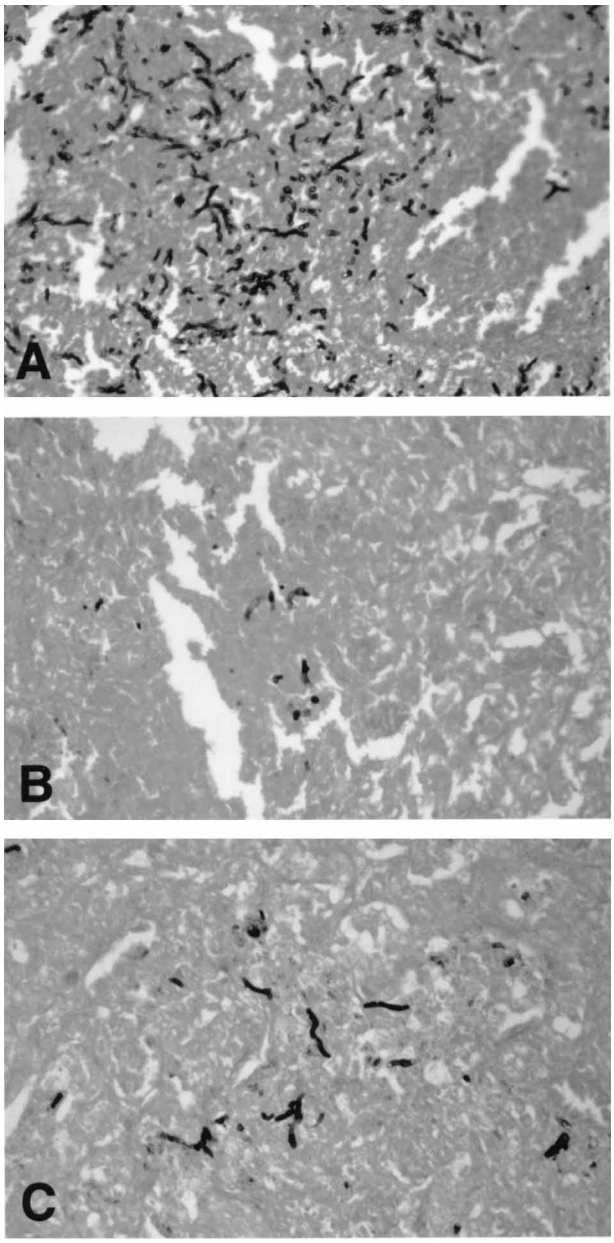

FIG. 3. Gomori methenamine silver stains of lung sections from control rats (A) and from rats treated with itraconazole (B) and voriconazole (C). Magnification, ×40.

In the rat model of IPA, voriconazole was better absorbed than itraconazole following oral administration of 30 mg/kg in corn oil. The maximum concentration of drug in serum was 4.56 ± 0.68 μg/ml for voriconazole, compared to 0.43 ± 0.23 μg/ml for itraconazole. In the first trial, a dose-response survival rate was observed in animals treated with voriconazole (Fig. 1). At 7 days postinfection, survival rates were 62.5, 75, and 100% for groups receiving 10, 20, and 30 mg of voriconazole per kg, respectively; the survival rate was 37.5% for

controls (for the group receiving voriconazole at 30 mg/kg versus the controls, $P < 0.02$). The survival rate was 100% for the group treated with high doses of amphotericin B ($P < 0.02$).

In the second trial, voriconazole and itraconazole were compared (at dosages of 30 mg/kg/day given orally) (Fig. 2). The survival rates were 100% for the voriconazole-treated group ($P < 0.01$) and 75% for the itraconazole-treated group ($P < 0.10$), compared to a survival rate of 41.6% for the control group. Representative histological sections of lung tissues from necropsied animals demonstrated abundant mycelia and extensive consolidation in control animals, but for animals treated with voriconazole or itraconazole only rare mycelia that were notable for their stunted, aberrant morphology were found (Fig. 3).

Voriconazole is a new broad-spectrum triazole antifungal agent with fungicidal activity against *Aspergillus* spp. (15). It appears to have good bioavailability and is well tolerated by humans (21), and early clinical studies of invasive aspergillosis have provided encouraging results (7, 9). In this study, we demonstrated that voriconazole has potent in vitro activity against a range of clinical isolates of *Aspergillus* spp. Voriconazole was well absorbed following oral administration and was highly effective in preventing or delaying mortality in an experimental model of pulmonary aspergillosis. Survival was greater with voriconazole treatment compared to that with itraconazole treatment, although this difference was not statistically significant. Previous studies with our model of IPA have validated its predictive value in assessing the efficacies of antifungal compounds for the treatment of pulmonary aspergillosis (17, 22, 23). Voriconazole is a promising agent for the treatment of this life-threatening disease of the immunocompromised host.

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