Activity of Posaconazole in Treatment of Experimental Disseminated Zygomycosis

Eric Dannaoui,^{1*} Jacques F. G. M. Meis,² David Loevenberg,³
and Paul E. Verweij¹

Department of Medical Microbiology, University Medical Center, St. Radboud, 6500 HB Nijmegen,¹
and Department of Medical Microbiology, Canisius-Wilhelmina Hospital, 65000 GS Nijmegen,²
The Netherlands, and Schering Plough Research Institute, Kenilworth, New Jersey 07033³

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Three isolates of zygomycetes were used to produce a disseminated infection in nonimmunocompromised mice. Against all zygomycete strains, amphotericin B significantly prolonged survival. Itraconazole was inactive against *Rhizopus microsporus* and *Rhizopus oryzae* but was partially active against *Absidia corymbifera*. Posaconazole had no beneficial effects against *R. oryzae* but showed partial activity against *A. corymbifera*. Posaconazole had a clear dose-response effect against *R. microsporus*.

Zygomycosis is a life-threatening infection occurring mostly in immunocompromised patients (19). Amphotericin B remains the first-line therapy, but its use is limited by toxicity. Moreover, despite amphotericin B therapy, mortality remains high particularly in disseminated zygomycosis (19), highlighting the need for new therapeutic strategies. It is generally assumed that there is no indication for the use of azole drugs in treating zygomycosis (11, 20). Nevertheless, it has been shown that some azoles alone (6) or in combination (21) had beneficial effects in animal models of *Rhizopus* infection. Itraconazole exhibited low MICs against some zygomycetes (2, 4, 9, 23, 25, 26), and recently, it was evaluated in murine models of zygomycosis (3, 14). Posaconazole is a triazole with broad-spectrum activity against yeasts (5, 18), dimorphic fungi (7, 12, 22), and filamentous fungi (1, 10, 17), including zygomycetes (2, 13, 23, 26). Posaconazole was active in vivo against *Mucor* spp. in a murine model of disseminated infection (24). The in vivo activity of this drug against other species of zygomycetes is unknown.

The aim of this study was to evaluate the in vivo efficacy of posaconazole against three strains of zygomycetes that belong to different species.

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Three strains were used in this study, *Rhizopus microsporus* var. *rhizopodiformis* AZN 1185, *Rhizopus oryzae* AZN 6373, and *Absidia corymbifera* AZN 4095. Susceptibility testing was performed by a National Committee for Clinical Laboratory Standards (NCCLS)-based broth microdilution technique (15) as previously described (3).

Female CD-1 mice (Harlan, Horst, The Netherlands), weighing 20 to 22 g, were used in these experiments. Inocula were prepared as spore suspensions in saline, counted microscopically, and adjusted to the required concentration. The

90% lethal doses were 7×10^6 and 7.5×10^5 CFU/mouse for *R. microsporus* and *A. corymbifera* (3), respectively, and 7.6×10^6 for *R. oryzae*. Mice were given the 90% lethal dose by injection into a tail vein.

Amphotericin B (Fungizone) was given in 5% glucose intraperitoneally. Itraconazole (Trisporal oral solution) was diluted in water and given by gavage. Posaconazole was prepared in methylcellulose (1) and given by gavage. Treatment was begun 2 h after infection and continued for 10 days. In each experiment, groups of 10 mice were used. One group was treated with 1 mg of amphotericin B per kg of body weight once daily (OD), one group was treated with 50 mg of itraconazole per kg twice a day (BID), and three groups were treated with posaconazole at 5, 25, or 40 mg/kg OD. Each experiment was run once. An additional experiment was conducted to evaluate posaconazole at 100 mg/kg OD in mice infected with *A. corymbifera*. Control mice received either 5% glucose intraperitoneally or methylcellulose by gavage. Mortality was recorded up to 5 days after the end of treatment. Organ infection was assessed by microscopic examination and qualitative cultures (3). Mortality data were compared by the log rank test. Microscopic examination results (presence of hyphae in organs) were compared by Fisher's exact test.

The susceptibilities of the three zygomycete isolates are shown in Table 1. Survival curves (Fig. 1) demonstrated that all three isolates caused an acute and lethal infection in control mice. Mice infected with *R. microsporus* (Fig. 1A) and treated with 1 mg of amphotericin B per kg had a 100% survival rate which was higher than the survival of untreated mice ($P = 0.0003$). All mice treated with itraconazole died by day 6, which was not significantly different from control mice. There was a dose-response effect for posaconazole. Posaconazole at 5 mg/kg did not prolong survival of the mice compared to the control mice. In contrast, 30% of mice treated with posaconazole at 25 mg/kg survived ($P = 0.0057$) and 100% of mice treated with posaconazole at 40 mg/kg survived ($P < 0.0001$).

Figure 1B shows that all control mice infected with *R. oryzae* died within 5 days. No mice treated with amphotericin B died, which was significantly different from the number of control

* Corresponding author. Present address: National Reference Center for Mycoses, Molecular Mycology Unit, Pasteur Institute, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France. Phone: 33 1 40 61 32 50. Fax: 33 1 45 68 84 20. E-mail: dannaoui@pasteur.fr.

TABLE 1. In vitro susceptibilities of three zygomycete isolates used in in vivo studies to amphotericin B, itraconazole, and posaconazole^a

Isolate	MIC ($\mu\text{g/ml}$)		
	Amphotericin B	Itraconazole	Posaconazole
<i>R. microsporus</i> AZN 1185	0.25	>16	1
<i>R. oryzae</i> AZN 6373	1	0.5	0.25
<i>A. corymbifera</i> AZN 4095	0.06	0.03	0.12

^a MICs of amphotericin B and itraconazole against *R. microsporus* and *A. corymbifera* are from reference 3.

mice that died ($P < 0.0001$). All mice treated with itraconazole or posaconazole died within 6 days.

Ninety percent of control mice infected with *A. corymbifera* died within 8 days (Fig. 1C). No mice treated with amphotericin B died, which was better than no therapy ($P < 0.0001$), and treatment with itraconazole, with 60% survival, prolonged sur-

vival compared to that of control mice ($P = 0.0015$). Groups treated with 5 or 25 mg of posaconazole per kg had a 30% survival rate, and 50% of mice that received posaconazole at 40 mg/kg survived, which was not significantly better than controls. In an additional experiment (Fig. 1D), 70% of mice infected with *A. corymbifera* and treated with posaconazole at 100 mg/kg survived ($P = 0.0239$).

Table 2 summarizes the results of cultures and microscopic examinations of brain and kidney samples. For each isolate, an active infection (presence of hyphae in tissues) was noted in the kidneys and brains of almost all control animals. Culture results did not always correlate with the presence of hyphae. In particular, in mice infected with *A. corymbifera*, only a small number of infected brains were positive by culture.

In mice infected with *R. microsporus*, amphotericin B reduced the number of kidneys with active infection ($P < 0.0001$) compared to the number for control mice. However, amphotericin B was not curative, as all cultures were positive, due to

FIG. 1. Cumulative mortality for mice infected with *R. microsporus* AZN 1185 (A), *R. oryzae* AZN 6373 (B), and *A. corymbifera* AZN 4095 (C and D) in treated and control groups. Symbols: ▲, itraconazole at 50 mg/kg BID; ■, amphotericin B at 1 mg/kg OD; ▼, posaconazole at 5 mg/kg OD; ▽, posaconazole at 25 mg/kg OD; ●, posaconazole at 40 mg/kg OD; ◇, posaconazole at 100 mg/kg OD; □, control, 5% glucose intraperitoneally; △, control, methylcellulose per os.

TABLE 2. Microscopic examination and culture results for control and treated mice infected with *R. microsporus*, *R. oryzae*, and *A. corymbifera*

Strain and group ^a (no. of mice in group)	No. of survivors	% of positive organs ^b					
		Kidney			Brain		
		Microscopic examination		Culture	Microscopic examination		Culture
		Hyphae	Spores		Hyphae	Spores	
<i>R. microsporus</i> AZN 1185							
Amphotericin B (10)	10	0 ^d	100	100	50	40	100
Itraconazole (10)	0	100	70	100	100	30	100
Posaconazole 5 (10)	0	100	10	100	90	40	100
Posaconazole 25 (10)	3	90	30	100	70	30	100
Posaconazole 40 (10)	10	100	0	100	90	20	100
Control i.p. (10)	2	100	10	100	78 ^c	33 ^c	100 ^c
Control p.o. (10)	0	100	10	100	90	30	100
<i>R. oryzae</i> AZN 6373							
Amphotericin B (10)	10	0 ^d	10	100	0 ^d	0	90
Itraconazole (10)	0	100	100	100	100	0	100
Posaconazole 5 (10)	0	100	90	100	100	0	100
Posaconazole 25 (10)	0	100	100	100	100	0	100
Posaconazole 40 (10)	0	100	100	100	100	0	100
Control i.p. (10)	0	100	90	100	100	0	100
Control p.o. (10)	0	100	80	100	100	0	100
<i>A. corymbifera</i> AZN 4095							
Amphotericin B (10)	10	0 ^d	0	30	0 ^d	0	0
Itraconazole (10)	6	50 ^e	0	70	70	0	0
Posaconazole 5 (10)	3	90	20	90	70	0	0
Posaconazole 25 (10)	3	80	50	80	70	0	10
Posaconazole 40 (10)	5	50 ^e	40	80	60	0	0
Control i.p. (10)	1	90	30	90	80	0	20
Control p.o. (10)	1	90	10	90	80	0	20

^a Amphotericin B was given at 1 mg/kg/day. Itraconazole was given at 50 mg/kg BID. Posaconazole was given at 5, 25, or 40 mg/kg/day. Controls received either 5% glucose intraperitoneally (i.p.) or methylcellulose per os (p.o.) by gavage.

^b Data from both control groups were pooled before statistical analysis.

^c The brain from one mouse was not tested.

^d Significantly different from the values obtained for the control groups ($P < 0.0001$).

^e Significantly different from the values obtained for the control groups ($P < 0.05$).

ungerminated spores in tissues. Itraconazole and posaconazole did not significantly reduce the number of positive organs from that of the controls.

Against *R. oryzae*, amphotericin B cleared active infections in both kidneys and brains ($P < 0.0001$). Itraconazole and posaconazole were not active in reducing the number of infected organs.

In mice infected with *A. corymbifera*, amphotericin B cleared active infections in organs ($P < 0.0001$). Itraconazole and posaconazole at 40 mg/kg significantly reduced the number of infected kidneys ($P < 0.05$).

Although amphotericin B is the treatment of choice for zygomycosis, its efficacy remains poor. The other antifungal agents, including the azoles, are considered ineffective (11, 20). Nevertheless, in vitro susceptibility data have been limited to a small number of isolates, and there have been few in vivo studies in animal models (3, 6, 8, 14, 16, 21, 24, 27, 28). Moreover, zygomycetes constitute a heterogeneous group for antifungal susceptibility (2). Of the new azoles, posaconazole possesses broad-spectrum activity against filamentous fungi, including zygomycetes (2, 13, 23, 26). Recently, posaconazole was shown to be effective in a model of *Mucor* infection (24).

In this study, indices used to assess the outcome of infection were the survival of animals and the percentage of infected

organs. As coenocytic hyphae can be damaged by the homogenization procedure applied to the organs, direct examinations (to demonstrate an active infection) were performed along with cultures (3).

R. oryzae is the most common microorganism that causes zygomycosis in humans (19). In the present study, amphotericin B was the only drug that showed in vivo activity against *R. oryzae*. Nevertheless, cultures showed that spores remained viable in the tissues. These results are in accordance with previous studies that showed that amphotericin B prolonged survival in infected animals but did not clear the fungus from the organs (16, 28). The two azoles tested in this study were not effective in vivo against *R. oryzae* despite their relatively low MICs, and similar results have been reported for itraconazole (16, 28).

Itraconazole was not active in mice infected with *R. microsporus* as shown previously (3). In contrast, a dose-effect relationship was observed for posaconazole. At 40 mg/kg, posaconazole was as effective as amphotericin B at 1 mg/kg in prolonging survival, but active infections in organs were less frequent in mice treated with amphotericin B. In a recent study, it has been shown that posaconazole increased the survival of mice infected with *Mucor* spp., while itraconazole was not active (24). Interestingly, in that study, in vivo efficacy was

not correlated with in vitro data, as the MIC of posaconazole for one species (*Mucor circinelloides*) was high (8 µg/ml).

A. corymbifera is another zygomycete pathogenic in humans (19). In our animal model, itraconazole was only partially active, although a low MIC was obtained against this isolate. Similarly, in a murine model of *A. corymbifera* infection, itraconazole, although less active than amphotericin B, increased the rate of survival of infected animals (14). In vivo efficacy of posaconazole showed a dose response, and posaconazole (100 mg/kg/day) significantly prolonged survival over that of the controls. It should be noted that untreated mice infected with *A. corymbifera* developed a very acute disease with early mortality 48 h after infection and a median survival time of 3 days. In contrast, for control mice infected with *R. microsporus*, death did not occur before day 5 postinfection. These differences could explain, at least in part, the higher activity of posaconazole against *R. microsporus*.

In conclusion, the results of this and other studies showed that posaconazole is effective against some species of zygomycetes. Clinical studies with posaconazole alone or in combination with other antifungal drugs are warranted.

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