ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2008, p. 1580–1582 Vol. 52, No. 4
0066-4804/08/$08.00+0 doi:10.1128/AAC.01384-07

In Vitro Activity of the New Azole Isavuconazole (BAL4815) Compared with Six Other Antifungal Agents against 162 *Cryptococcus neoformans* Isolates from Cuba▽

Maria-Teresa Illnait-Zaragozi,[1,2] Gerardo F. Martínez,[2] Ilse Curfs-Breuker,[1] Carlos M. Fernández,[2] Teun Boekhout,[3] and Jacques F. Meis[1]*

Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands[1]; Department of Medical Microbiology, Laboratory of Mycology, Institute of Tropical Medicine Pedro Kouri, Havana, Cuba[2]; and Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands[3]

Received 26 October 2007/Returned for modification 19 November 2007/Accepted 11 January 2008

Cuban *Cryptococcus* isolates ($n = 165$) were tested in vitro against amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole, giving MIC_{90} values of 0.25, 8, 4, 0.25, 0.125, 0.016, and 0.016 μg/ml, respectively. Isavuconazole and posaconazole seem to be potentially active drugs for treating cryptococcal infections.

Antifungals available for therapy of cryptococcosis are limited to amphotericin B, flucytosine, and fluconazole; however, the side effects associated with administration of amphotericin B and flucytosine may restrict their use (17, 25). A new generation of triazoles, including posaconazole, voriconazole, ravuconazole, and isavuconazole, has been developed. These agents possess potent broad-spectrum activity and favorable pharmacokinetic profiles (14, 18, 23). Isavuconazole is a water-soluble triazole that is suitable for oral and intravenous administration; its active moiety, BAL4815, is a potent inhibitor of ergosterol biosynthesis. In vitro, the active drug shows activity against all major opportunistic and pathogenic fungi (14, 20, 21, 23).

Previous studies have been done regarding the activity of the new azoles against a wide variety of fungi. This report summarizes the in vitro activities of isavuconazole and six other antifungal drugs on a large number of Cuban *Cryptococcus* isolates from clinical and environmental origins by a broth microdilution method in accordance with the CLSI (formerly NCCLS) M27-A2 guidelines (12). To our knowledge, this is the first report evaluating the susceptibility of this yeast to this new triazole and the largest study ever done with Cuban *Cryptococcus* strains.

(Part of this work was presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 27 to 30 September 2006 [10a].)

A total of 165 *Cryptococcus* strains were included: 117 were obtained from clinical samples, including one isolated from a cheetah imported from South Africa. Forty-five strains were isolated from pigeon guano in three different geographical locations of Cuba: Havana City ($n = 8$), Cienfuegos ($n = 6$), and Pinar del Río ($n = 31$). Species identification was initially performed by standard mycological methods (9), and samples were stored on sterile water at room temperature until the study was carried out. Before use, the identities of all isolates were confirmed with a commercial identification system (Auxacolor 2; Bio-Rad, Marnes-la-Coquette, France). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 strains were used for quality control.

Standard antifungal powders of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole were used and were obtained from Sigma (The Netherlands), Valeant Pharmaceuticals (The Netherlands), Pfizer Central Research (United Kingdom), Janssen-Cilag (The Netherlands), Pfizer Central Research (United States), Schering Plough (United States), and Basilea Pharmaceutica (Switzerland), respectively. The stock solutions of the drugs were prepared in the appropriate solvent (12).

Broth microdilution testing was performed in accordance with the guidelines in CLSI document M27-A2 (12). The final concentrations of the antifungal agents were 0.016 to 8 μg/ml for amphotericin B, itraconazole, voriconazole, and posaconazole; 0.063 to 32 μg/ml for flucytosine and fluconazole; and 0.004 to 4.00 μg/ml for isavuconazole. Drug-free and yeast-free controls were included. The MICs at 48 and 72 h were read optically and spectrophotometrically at 420 nm after agitation. The MIC was defined as the lowest concentration of drug showing no growth for amphotericin B and a prominent reduction of growth (≥50%) for the other antifungals compared to the drug-free growth control.

All strains were identified as *Cryptococcus neoformans* var. *grubii*, except one obtained from an animal (*Cryptococcus gattii*) and two obtained from environmental samples from Pinar del Rio (*Cryptococcus albidus* and *Cryptococcus albidisimilus*). No differences between visual and spectrophotometric readings were observed, and the MICs for the quality control strains were all within the reference ranges (data not shown). Table 1 summarizes the in vitro susceptibilities of all the isolates according to the origin of the strain. In this study, most of the *C. neoformans* isolates showed quite uniform patterns of susceptibility to the antifungal agents tested. All of the strains

* Corresponding author. Mailing address: Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, P.O. Box 9015, 6500 GS Nijmegen, The Netherlands. Phone: 31243657514. Fax: 31243657516. E-mail: j.meis@cwz.nl.

▽ Published ahead of print on 22 January 2008.

TABLE 1. In vitro susceptibilities of *Cryptococcus* isolates to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole according to origin and underlying disease of patients

Origin (no. of strains)	Antifungal agent	MIC (μg/ml)		
		Range	50%	90%
Environmental (45)	Amphotericin B	0.016–0.5	0.25	0.5
	Flucytosine	0.5–64	4	8
	Fluconazole	0.25–64	4	8
	Itraconazole	0.016–0.5	0.063	0.25
	Voriconazole	0.016–0.25	0.063	0.25
	Posaconazole	0.016–0.125	0.31	0.63
	Isavuconazole	0.002–0.031	0.004	0.016
Clinical (117)	Amphotericin B	0.031–1	0.25	0.25
	Flucytosine	0.063–8	4	8
	Fluconazole	0.25–8	2	4
	Itraconazole	0.016–1	0.031	0.25
	Voriconazole	0.016–0.25	0.031	0.125
	Posaconazole	0.016–0.5	0.004	0.016
	Isavuconazole	0.002–0.063	0.004	0.016
AIDS patients (85)	Amphotericin B	0.031–1	0.25	0.25
	Flucytosine	0.125–8	4	8
	Fluconazole	0.25–8	2	2
	Itraconazole	0.016–0.5	0.031	0.125
	Voriconazole	0.016–0.25	0.031	0.125
	Posaconazole	0.016–0.125	0.031	0.125
	Isavuconazole	0.002–0.063	0.008	0.016
Non-AIDS patients (32)	Amphotericin B	0.125–1	0.25	1
	Flucytosine	0.063–8	2	4
	Fluconazole	0.25–8	2	8
	Itraconazole	0.016–1	0.031	0.063
	Voriconazole	0.016–0.25	0.063	0.063
	Posaconazole	0.016–0.5	0.063	0.125
	Isavuconazole	0.004–0.063	0.008	0.031
Total	Amphotericin B	0.016–1	0.25	0.25
	Flucytosine	0.063–64	4	8
	Fluconazole	0.25–64	2	4
	Itraconazole	0.016–1	0.031	0.25
	Voriconazole	0.016–0.25	0.031	0.125
	Posaconazole	0.002–0.125	0.004	0.016
	Isavuconazole	0.002–0.063	0.004	0.016

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

were susceptible to amphotericin B, voriconazole, posaconazole, and isavuconazole.

When all of the strains were considered together, the widest ranges and highest MICs were for flucytosine (0.063 to 64 μg/ml) and fluconazole (0.25 to 64 μg/ml). The lowest MICs were for isavuconazole, voriconazole, and posaconazole.

The environmental isolates seem to be less susceptible to fluconazole than the clinical ones.

Strains obtained from human immunodeficiency virus-negative patients showed lower MIC_{90}s, especially for amphotericin B and fluconazole, compared with those from AIDS patients.

Our results on the in vitro activities of the main antifungal drugs against *C. neoformans* are similar to those published previously (3, 7, 8, 16, 19, 24). Amphotericin B has long successfully been used to treat various yeast and mold infections. Unfortunately, its clinical use is hindered by intrinsic nephrotoxicity (5). There are no defined amphotericin B breakpoints by CLSI for *C. neoformans.* For this reason, breakpoints were used according to Nguyen and Yu (13) and Lozano et al. (11). They define an isolate as resistant with a MIC of ≥2 μg/ml, which was found to be associated with therapeutic failure. Although isolates from non-HIV patients showed higher amphotericin B MICs, the studied strains appear to be susceptible to this drug.

Flucytosine is a drug with a limited spectrum of action that includes *Candida* spp. and *C. neoformans* (5). The CLSI M27-A2 document recommends that isolates for which MICs are 32 μg/ml be regarded as resistant to this drug (12). By this definition, none of the studied clinical isolates was resistant; nevertheless, a justified fear of the emergence of resistance has led to its use in combination with amphotericin B in vitro and in patients with cryptococcosis (4, 7, 22, 25).

Among the azoles, fluconazole showed the lowest activity. In fact, previous reports have already demonstrated the low activity of this drug against *C. neoformans* isolates, even though it has proven to be more active in vivo (1). According to these authors, the good therapeutic results obtained are largely attributable to its high concentrations in cerebrospinal fluid. Although no resistance has been found in the present collection of isolates, we continue paying attention to the emerging resistance to this antifungal agent due to its widespread use as primary prophylaxis in AIDS patients.

Fortunately, the antifungal armamentarium has increased during the past two decades with the addition of several agents and, although the echinocandins seem to be inactive, other new triazoles are expected to be licensed shortly (5, 14). Voriconazole has in vitro activity against cryptococcal yeasts (15) including those that are resistant in vitro to fluconazole (13). Our results show that voriconazole was more potent than itraconazole against Cuban *Cryptococcus* isolates. However, these promising in vitro results should be complemented by clinical confirmation.

Posaconazole and isavuconazole belong to the latest generation of azole antifungal agents that are being investigated for their role in treating serious infections due to yeasts and molds. In addition to potent activity, they are well tolerated and offer a diminished toxicity profile compared with other currently marketed systemic antimycotics and their pharmacokinetics are characterized by slow elimination, low plasma clearance, and extensive tissue distribution (14, 26, 27).

Posaconazole is the broadest-spectrum azole licensed to date. It exhibits linear pharmacokinetics in volunteers (6) and has demonstrated safety and efficacy comparable to those of fluconazole in human and animal studies, although there is no intravenous formulation available (26). In agreement with our results with *Cryptococcus*, other in vitro studies have documented a potency and spectrum of activity against clinically important yeasts and *Aspergillus* spp. similar to those of itraconazole and superior to those of fluconazole. Consistent with these results, in vivo studies have demonstrated efficacy in treating infections due to *Candida* spp. and *C. neoformans* with this antifungal agent (2, 10).

The prodrug isavuconazole is a water-soluble triazole precursor that is suitable for oral and intravenous administration. Its active moiety, BAL4815, is a potent inhibitor of ergosterol biosynthesis (14, 20, 21). In vitro, the drug shows broad-spectrum activity against all major opportunistic and true patho-

genic fungi (14, 23). In animals as well as in humans, the pharmacokinetics of this drug are characterized by slow elimination, low plasma clearance (approximately 10% of liver blood flow), and extensive tissue distribution (20, 21). To our knowledge, this is the first report evaluating the action against *Cryptococcus* spp. of isavuconazole, which seems to be the most effective antifungal drug in terms of in vitro activity. In conclusion, our results indicate that there has been no significant shift in the MICs of amphotericin B and fluconazole for Cuban *C. neoformans* (8), despite the widespread use of these agents in persons with AIDS. Given the high oral bioavailability and the well-tolerated nature of the new azoles, they might become an important addition to the armamentarium of antifungal agents: both voriconazole and posaconazole show strong antifungal activity against Cuban *Cryptococcus* strains in vitro, while isavuconazole showed even lower MICs. These promising results still need to be correlated with clinical outcome.

This study was made possible by an unrestricted research grant from the International Society of Human and Animal Mycology (ISHAM) to M.T.I.-Z.

REFERENCES

1. **Aller, A. I., E. Martin-Mazuelos, F. Lozano, J. Gomez-Mateos, L. Steele-Moore, W. J. Holloway, M. J. Gutiérrez, F. J. Recio, and A. Espinel-Ingroff.** 2000. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. Antimicrob. Agents Chemother. **44:**1544–1548.
2. **Barchiesi, F., E. Spreghini, A. M. Schimizzi, M. Maracci, D. Giannini, F. Carle, and G. Scalise.** 2004. Posaconazole and amphotericin B combination therapy against *Cryptococcus neoformans* infection. Antimicrob. Agents Chemother. **48:**3312–3316.
3. **Brandt, M. E., M. A. Pfaller, R. A. Hajjeh, R. J. Hamill, P. G. Pappas, A. L. Reingold, D. Rimland, and D. W. Warnock for The Cryptococcal Disease Active Surveillance Group.** 2001. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates in the United States: 1992 to 1994 and 1996 to 1998. Antimicrob. Agents Chemother. **45:**3065–3069.
4. **Brouwer, A. E., A. Rjanuwong, W. Chierakul, G. E. Griffin, R. A. Larsen, N. J. White, and T. S. Harrison.** 2004. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet **363:** 1764–1767.
5. **Chen, S. C., and T. C. Sorrell.** 2007. Antifungal agents: new drugs, old drugs. Med. J. Aust. **187:**404–409.
6. **Courtney, R., S. Pai, M. Laughlin, J. Lim, and V. Batra.** 2003. Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults. Antimicrob. Agents Chemother. **47:** 2788–2795.
7. **Dannaoui, E., M. Abdul, M. Arpin, A. Michel-Nguyen, M. A. Piens, A. Favel, O. Lortholary, and F. Dromer for the French Cryptococcosis Study Group.** 2006. Results obtained with various antifungal susceptibility testing methods do not predict early clinical outcome in patients with cryptococcosis. Antimicrob. Agents Chemother. **50:**2464–2470.
8. **Fernández, C. M., M. González, M. T. Illnait, and G. F. Martínez.** 1998. Determinación de la concentración mínima inhibitoria de anfotericina B en levaduras de interés médico. Rev. Cuba. Med. Trop. **50:**48–53.
9. **Hazen, K. C., and S. A. Howell.** 2003. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 1693–1711. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed., vol. 2. ASM Press, Washington, DC.
10. **Herbrecht, R., Y. Nivoix, C. Fohrer, S. Natarajan-Ame, and V. Letscher-Bru.** 2005. Management of systemic fungal infections: alternatives to itraconazole. J. Antimicrob. Chemother. **56:**39–48.

10a. **Illnait-Zaragozi, M., I. Curfs-Breuker, G. Martinez, C. Fernandez, T. Boekhout, and J. Meis.** Abstr. 46th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1587, p. 420.

11. **Lozano-Chiu, M., V. L. Paetznick, M. A. Ghannoum, and J. H. Rex.** 1998. Detection of resistance to amphotericin B among *Cryptococcus neoformans* clinical isolates: performances of three different media assessed by using E-test and National Committee for Clinical Laboratory Standards M27-A methodologies. J. Clin. Microbiol. **36:**2817–2822.
12. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.
13. **Nguyen, M. H., and C. Y. Yu.** 1998. In vitro comparative efficacy of voriconazole and itraconazole against fluconazole-susceptible and -resistant *Cryptococcus neoformans* isolates. Antimicrob. Agents Chemother. **42:**471–472.
14. **Odds, F. C.** 2006. Drug evaluation: BAL-8557, a novel broad-spectrum triazole antifungal. Curr. Opin. Investig. Drugs **7:**766–772.
15. **Perfect, J. R., K. A. Marr, T. J. Walsh, R. N. Greenberg, B. Dupont, J. Torres-Cisneros, G. Just-Nubling, H. T. Schlamm, I. Lutsar, A. Espinel-Ingroff, and E. Johnson.** 2003. Voriconazole treatment for less-common, emerging, or refractory fungal infections. Clin. Infect. Dis. **36:**1122–1131.
16. **Pfaller, M. A., S. A. Messer, L. Boyken, R. J. Hollis, C. Rice, S. Tendolkar, and D. J. Diekema.** 2004. In vitro activities of voriconazole, posaconazole, and fluconazole against 4,169 clinical isolates of *Candida* spp. and *Cryptococcus neoformans* collected during 2001 and 2002 in the ARTEMIS global antifungal surveillance program. Diagn. Microbiol. Infect. Dis. **48:**201–205.
17. **Saag, M. S., J. R. Graybill, R. A. Larsen, P. G. Pappas, J. R. Perfect, W. G. Powderky, J. D. Sobel, and W. E. Dismukes.** 2000. Practice guidelines for the management of cryptococcus disease. Clin. Infect. Dis. **30:**710–718.
18. **Sabatelli, F., R. Patel, P. A. Mann, C. A. Mendrick, C. C. Norris, R. Hare, D. Loebenberg, T. A. Black, and P. M. McNicholas.** 2006. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. Antimicrob. Agents Chemother. **50:**2009–2015.
19. **Salam, A. H. A.** 2005. In vitro susceptibility of *Cryptococcus neoformans* clinical isolates from Egypt to seven antifungal drugs. Mycoses **48:**327–332.
20. **Schmitt-Hoffmann, A., B. Roos, M. Heep, M. Schleimer, E. Weidekamm, T. Brown, M. Roehrle, and C. Beglinger.** 2006. Single-ascending-dose pharmacokinetics and safety of the novel broad-spectrum antifungal triazole BAL4815 after intravenous infusions (50, 100, and 200 milligrams) and oral administrations (100, 200, and 400 milligrams) of its prodrug, BAL8557, in healthy volunteers. Antimicrob. Agents Chemother. **50:**279–285.
21. **Schmitt-Hoffmann, A., B. Roos, J. Maares, M. Heep, J. Spickerman, E. Weidekamm, T. Brown, and M. Roehrle.** 2006. Triazole BAL4815 after intravenous infusion and oral administration of its prodrug, BAL8557, in healthy volunteers. Antimicrob. Agents Chemother. **50:**286–293.
22. **Schwarz, P., G. Janbon, F. Dromer, O. Lortholary, and E. Dannaoui.** 2007. Combination of amphotericin B with flucytosine is active in vitro against flucytosine-resistant isolates of *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **51:**383–385.
23. **Seifert, H., U. Aurbach, D. Stefanik, and O. Cornely.** 2007. In vitro activities of isavuconazole and other antifungal agents against *Candida* bloodstream isolates. Antimicrob. Agents Chemother. **51:**1818–1821.
24. **Souza, L. K., O. F. L. Fernandes, C. C. Kobayashi, X. S. Passos, C. R. Costa, J. A. Lemos, A. H. Souza-Júnior, and M. R. Silva.** 2005. Antifungal susceptibilities of clinical and environmental isolates of *Cryptococcus neoformans* in Goiânia City, Goiás, Brazil. Rev. Inst. Med. Trop. Sao Paulo **47:**253–256.
25. **te Dorsthorst, D. T. A., P. E. Verweij, J. Meletiadis, M. Bergervoet, N. C. Punt, J. F. Meis, and J. W. Mouton.** 2002. In vitro interaction of flucytosine combined with amphotericin B or fluconazole against thirty-five yeast isolates determined by both the fractional inhibitory concentration index and the response surface approach. Antimicrob. Agents Chemother. **46:**2982–2989.
26. **Torres, H. A., R. Y. Hachen, R. F. Chemaly, D. P. Kontoyiannis, and I. Raad.** 2005. Posaconazole: a broad-spectrum triazole antifungal. Lancet Infect. Dis. **5:**775–785.
27. **Ullmann, A. J., O. A. Cornely, A. Burchardt, R. Hachem, D. P. Kontoyiannis, K. Topelt, R. Courtney, D. Wexler, G. Krishna, M. Martinho, G. Corcoran, and I. Raad.** 2006. Pharmacokinetics, safety, and efficacy of posaconazole in patients with persistent febrile neutropenia or refractory invasive fungal infection. Antimicrob. Agents Chemother. **50:**658–666.