Mycopathologia (2006) 162: 111–114
DOI: 10.1007/s11046-006-0039-4

Efficacy of voriconazole in experimental *Cryptococcus neoformans* infection

Nikolaos Mavrogiorgos[1,3], Oscar Zaragoza[2], Arturo Casadevall[1,2] & Joshua D. Nosanchuk[1,2]
[1]*Departments of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA;* [2]*Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, 10461, USA;* [3]*Boston University Medical Center, Boston, MA, USA*

Received 7 April 2006; accepted 13 June 2006

Abstract

Voriconazole is a third generation triazole with improved activity against many fungal pathogens. We examined the efficacy of voriconazole in a murine infection model and evaluated the drug's effect on cellular characteristics and serum polysaccharide levels. The antifungal reduced serum polysaccharide and significantly prolonged the survival of lethally infected animals.

Key words: *Cryptococcus neoformans*, voriconazole, antifungal, polysaccharide capsule

Introduction

Voriconazole is a broad-spectrum triazole antifungal that inhibits cytochrome P450-dependent 14α-lanosterol demethylation, which is a critical step in fungal cell membrane ergosterol synthesis. Voriconazole is a synthetic derivative of fluconazole that differs from fluconazole by the replacement of one of the triazole rings with a fluorinated pyrimidine as well as the addition of an α-methyl group. *Cryptococcus neoformans* is a relatively frequent cause of serious fungal infections in individuals with HIV. The prevalence of cryptococcal meningo-encephalitis in individuals with AIDS in the United States is currently estimated to be less than 2% [1], but is over 30% in areas of South East Asia and Sub-Saharan Africa [2]. Patients with AIDS complicated by cryptococcosis often respond poorly to treatment, and they require lifelong maintenance therapy in the setting of continued immunosuppression since currently available antifungal agents seldom eradicate this pathogen [3]. Voriconazole demonstrates excellent in vitro activity against *C. neoformans* [4, 5] and achieves good CSF levels [6]. No clinical trials have evaluated the efficacy of voriconazole for cryptococcal disease and there is a paucity of published information regarding the clinical use of the drug for cryptococcosis [7–9]. In this study, we evaluated the activity of voriconazole in a lethal *C. neoformans* infection model.

C. neoformans var. *grubii* (serotype A) strain H99 (ATCC, Manassas, VA) was used for this study because it represents the most prevalent clinical serotype in the US [10]. The fungus was grown overnight in Sabouraud medium (30 °C, at 150 rpm) then collected by centrifugation, washed and suspended in PBS, and counted in a hemacytometer. A suspension of 2×10^7 cells/ml was prepared and 50 μl were injected intratracheally in female C57BL/6J mice (6–8 weeks old, National Cancer Institute, Bethesda, MD).

For assessment of fungal burden and cellular characteristics and determination of serum polysaccharide levels, mice were treated with voriconazole (Pfizer, Sandwich, England) beginning the

day after infection at 1, 5, 20, or 40 mg/kg/day in PEG400 (Sigma Chemical Corp., Cleveland, Ohio) orally by gavage ($n = 4$ mice per group). Infected control mice received PEG400. The mice were sacrificed at day 7 after infection. The lungs were removed and homogenized in 10 ml of PBS. Collagenase A was added at 10 mg/ml (Roche, IN), the extract was incubated for 90 minutes at 37 °C with vortexing, and the cells were washed with dH_2O to lyse mammalian cells. Aliquots of the cell preparations were plated onto Sabouraud agar in triplicate (1 colony = 1 CFU). India ink preparations were viewed with an Olympus AX70 (Melville, NY) microscope and images obtained QImaging Retiga 1300 digital camera (Burnaby BC, Canada) with QCapture Suite V2.46 software (QImaging). Capsule thickness was determined by subtracting the diameter of the cell body from that of the whole cell (capsule plus cell body). Four mice were sacrificed per group, and at least 200 cells were counted per mouse. Serum samples from the mice were also collected. The sera were treated with proteinase K (1 mg/ml, Roche, IN) then used in a capture ELISA to determine the quantity of circulating glucuronoxylomannan (GXM), the major component of *C. neoformans* polysaccharide [11]. GXM levels were calculated relative to H99 GXM standards [12].

The results of the studies performed on mice sacrificed 7 days post infection are summarized in Table 1. The experiments were repeated once with similar results. There was no statistically significant difference in the CFUs between the groups of mice or in the percentage of the capsule size of *C. neoformans* cells present in the lung homogenates. There were also no differences in the size of capsule or cell bodies (data not shown). In contrast, our prior studies with voriconazole and *C. neoformans* in vitro showed that voriconazole significantly reduced the size of both the capsule and the cell body [13]. However, homogeneity may benefit the host immune response during infection, since heterogeneity of *C. neoformans* cells has been linked to dissemination [14]. We did identify a trend towards decreased concentration of GXM in the serum of mice receiving increasing doses of voriconazole, though the differences were not statistically significant (P values = 0.30, 0.47, 0.13, and 0.14, respectively by Kruskal–Wallace) due to the large standard of error in the control sera. Interestingly, there was no difference in GXM released into liquid cultures of cells induced to produce large capsules by growth in DME and 10% fetal calf serum [13] that were subsequently incubated overnight in 10% CO_2 at 37 °C in the presence or absence of various concentrations of voriconazole (data not shown). The polysaccharide capsule is a major virulence factor of *C. neoformans* and GXM is a potent immunomodulator [15] and toxic to phagocytic cells [16]. Hence, a reduction of serum GXM despite the absence of a decrease of CFU one week after infection could have a dramatic impact on a host's ability to respond to the invading fungus.

For survival studies, mice received 10^6 CFU intratracheally and then were treated with 5 or 20 mg/kg/qD of voriconazole or PEG400 by gavage for 10 days beginning the day after infection ($n = 9$ mice per group). Kaplan–Meyer analysis showed that mice receiving voriconazole survived significantly longer than control animals (Figure 1), $P < 0.01$. In a second survival experiment ($n = 6$ mice per group) testing voriconazole at 1, 5, 20, or 40 mg/kg/QD, all doses provided a

Table 1. CFU, percentage of capsule comprising the yeast cells, and serum GXM levels from mice infected with *C. neoformans* for 7 days

Treatment	CFU $\times 10^{6}$ [a,b]	% Capsule[c]	GXM level[d]
PEG400	5.4 ± 2.6	60.5 ± 8.9	15.6 ± 18
Vori 1 mg	5.1 ± 1.2	53.6 ± 15.1	7.3 ± 6.9*
Vori 5 mg	5.5 ± 3.5	69.9 ± 8.8	11.2 ± 9.6*
Vori 20 mg	6.6 ± 2.2	65.8 ± 7.6	1.5 ± 0.7*
Vori 40 mg	5.7 ± 1.6	62.1 ± 23.2	1.9 ± 1.4*

[a]1 colony = 1 CFU.
[b]Values in all columns are averages + /− the standard errors.
[c]% capsule is determined by (capsule thickness/total cell) × 100
[d]Serum GXM in μg/ml.
*$P < 0.05$ for PEG400 versus voriconazole.

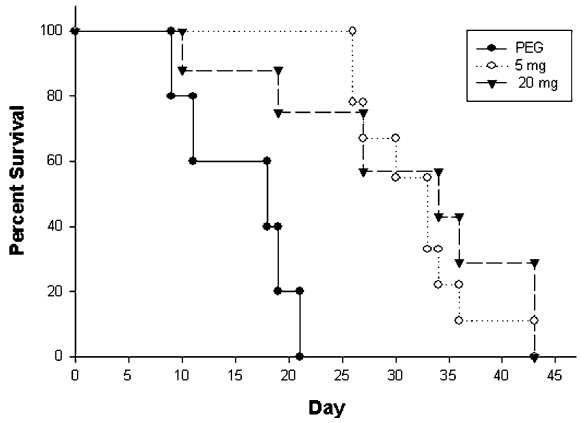

Figure 1. Kaplan–Meier plots showing survival of mice with pulmonary cryptococcal infection after treatment with 5 or 20 mg/kg/QD of voriconazole or placebo (PEG), $P < 0.01$ for both voriconazole doses compared to controls.

significant survival advantage (median survival of controls: 11 days; 1 mg/kg: 21 days, $P = 0.02$; 5 mg/kg: 32 days, $P = 0.02$; 20 mg/kg: 25 days, $P = 0.04$; 40 mg/kg: 32 days, $P = 0.01$).

These studies show that voriconazole is effective against *C. neoformans* in vivo. Since the fungal burden does not appear to be significantly impacted early in infection by voriconazole, the protective effects may be in part due to alterations in the host inflammatory response. Alternatively, the lack of difference could be due to the autoinduction of the metabolism of voriconazole that occurs in mice, rats, and dogs [17]. Steady-state plasma concentrations remain stable over time in humans [18]. Future studies in a guinea pig or rabbit model, where autoinduction does not occur, may provide additional insights to this issue. Nevertheless, the reduction in serum GXM that occurred with voriconazole administration could result in an improved host response to the fungus. These findings support the use of voriconazole in human cryptococcal infections and suggest future avenues of research into host responses to the fungus with and without voriconazole treatment.

References

1. Mirza SA, Phelan M, Rimland D, Graviss E, Hamill R, Brandt ME et al. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992–2000. Clin. Infect Dis 2003; 36(6): 789–794.
2. Perfect JR, Casadevall A. Cryptococcosis. Infect Dis Clin North Am 2002; 16(4):837–874, v–vi.
3. Zuger A, Louie E, Holzman RS, Simberkoff MS, Rahal JJ. Cryptococcal disease in patients with the acquired immunodeficiency syndrome: diagnostic features and outcome of treatment. Ann Intern Med 1986; 104: 234–240.
4. Hoban DJ, Zhanel GG, Karlowsky JA. In vitro susceptibilities of *Candida* and *Cryptococcus neoformans* isolates from blood cultures of neutropenic patients. Antimicrob Agents Chemother. 1999; 43(6): 1463–1464.
5. Pfaller MA, Zhang J, Messer SA, Brandt ME, Hajjeh RA, Jessup CJ et al. In vitro activities of voriconazole, fluconazole, and itraconazole against 566 clinical isolates of *Cryptococcus neoformans* from the United States and Africa. Antimicrob. Agents Chemother. 1999; 43(1): 169–171.
6. Schwartz S, Milatovic D, Thiel E. Successful treatment of cerebral aspergillosis with a novel triazole (voriconazole) in a patient with acute leukaemia. Br J Haematol 1997; 97(3): 663–665.
7. Friese G, Discher T, Fussle R, Schmalreck A, Lohmeyer J. Development of azole resistance during fluconazole maintenance therapy for AIDS-associated cryptococcal disease. AIDS 2001; 15(17): 2344–2345.
8. Perfect JR, Marr KA, Walsh TJ, Greenberg RN, DuPont B, Torre-Cisneros Jde la et al. Voriconazole treatment for less-common, emerging, or refractory fungal infections. Clin Infect Dis 2003; 36(9): 1122–1131.
9. Sabbatani S, Manfredi R, Pavoni M, Consales A, Chiodo F. Voriconazole proves effective in long-term treatment of a cerebral cryptococcoma in a chronic nephropathic HIV-negative patient, after fluconazole failure. Mycopathologia 2004; 158(2): 165–171.
10. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. J Clin Microbiol 1999; 37(3): 838–840.
11. Cherniak MR, Sundstrom JB. Polysaccharide antigens of the capsule of *Cryptococcus neoformans*. Infect Immunol 1994; 62: 1507–1512.
12. Casadevall A, Mukherjee J, Scharff MD. Monoclonal antibody based ELISA for cryptococcal polysaccharide. J. Immunol. Methods 1992; 154: 27–35.

13. Duin DVan, Cleare W, Zaragoza O, Casadevall A, Nosanchuk JD. Effects of voriconazole on *Cryptococcus neoformans*. Antimicrob. Agents Chemother. 2004; 48(6): 2014–2020.
14. Charlier C, Chretien F, Baudrimont M, Mordelet E, Lortholary O, Dromer F. Capsule structure changes associated with *Cryptococcus neoformans* crossing of the blood-brain barrier. Am. J. Pathol. 2005; 166(2): 421–432.
15. Vecchiarelli A. Immunoregulation by capsular components of *Cryptococcus neoformans*. Med Mycol 2000; 38(6): 407–417.
16. Feldmesser M, Kress Y, Novikoff P, Casadevall A. *Cryptococcus neoformans* is a facultative intracellular pathogen in murine pulmonary infection. Infect Immun 2000; 68(7): 4225–4237.
17. Roffey SJ, Cole S, Comby P, Gibson D, Jezequel SG, Nedderman AN et al. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab Dispos 2003; 31(6): 731–741.
18. Leveque D, Nivoix Y, Jehl F, Herbrecht R. Clinical pharmacokinetics of voriconazole. Int J Antimicrob. Agents 2006; 27(4): 274–284.

Address for Correspondence: Joshua D. Nosanchuk, Department of Medicine Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, 10461, USA
E-mail: nosanchu@aecom.yu.edu