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

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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 14\(\alpha\)-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 \(\alpha\)-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 \(2 \times 10^7\) cells/ml was prepared and 50 \(\mu\)l 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 \( (n = 4 \text{ mice per group}) \). 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 dH2O to lyse mammalian cells. Aliquots of the cell preparations were plated onto Sabouraud agar in triplicate \( (1 \text{ colony} = 1 \text{ CFU}) \). India ink preparations were viewed with an Olympus AX70 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 \( (\text{capsule plus cell body}) \). 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 \( (1 \text{ mg/ml, Roche, IN}) \) then used in a capture ELISA to determine the quantity of circulating glucuronoxylomannan \( (\text{GXM}) \), the major component of \( \text{C. neoformans} \) 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 \( \text{C. neoformans} \) cells present in the lung homogenates. There were also no differences in the size of capsule or cell bodies \( (\text{data not shown}) \). In contrast, our prior studies with voriconazole and \( \text{C. neoformans} \) 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 \( \text{C. neoformans} \) 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 \( (P \text{ values} = 0.30, 0.47, 0.13, \text{ and } 0.14, \text{ respectively by Kruskal–Wallace}) \) 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% \( \text{CO}_2 \) at 37 °C in the presence or absence of various concentrations of voriconazole \( (\text{data not shown}) \). The polysaccharide capsule is a major virulence factor of \( \text{C. neoformans} \) 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 \( 10^6 \text{ CFU} \) 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 \( (n = 9 \text{ mice per group}) \). Kaplan–Meier analysis showed that mice receiving voriconazole survived significantly longer than control animals (Figure 1), \( P < 0.01 \). In a second survival experiment \( (n = 6 \text{ mice per group}) \) testing voriconazole at 1, 5, 20, or 40 mg/kg/QD, all doses provided a

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

\(^a1\text{ colony} = 1 \text{ CFU.}\)

\(^b\text{Values in all columns are averages } +/− \text{ the standard errors.}\)

\(^c\% \text{ capsule is determined by } (\text{capsule thickness/total cell}) \times 100\)

\(^d\text{Serum GXM in } \mu\text{g/ml.}\)

\(^*P < 0.05 \text{ for PEG400 versus voriconazole.}\)