Anidulafungin Treatment of Candidal Central Nervous System Infection in a Murine Model

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Abstract

We established a murine model of *Candida albicans* central nervous system (CNS) infection and evaluated the efficacy of anidulafungin. Anidulafungin 10 mg/kg/day, amphotericin B, or voriconazole significantly reduced mortality and fungal burden in brain tissue, although amphotericin B and anidulafungin 10 mg/kg/day reduced fungal burden in brain tissue to a greater extent than did voriconazole. This suggests a potential role for anidulafungin in the treatment of candidal CNS infection.

Key words: *Candida albicans*; candidiasis; anidulafungin; echinocandin; central nervous system infection
Prolonged candidiasis can affect the central nervous system (CNS), inducing diffuse encephalopathy with microabscesses (9, 11). Hematogenous candidal meningoencephalitis is a relatively common and serious manifestation of disseminated candidiasis in premature infants (5). Echinocandins have excellent clinical activity in the treatment of invasive candidiasis (10, 15). Little is known regarding the activity of anidulafungin for the treatment of candidal CNS infection. We established a murine model of candidal CNS infection and evaluated the activity of anidulafungin in candidal CNS infection.

*Candida albicans* IDRL-5319 (a blood culture isolate), was prepared as previously described (14). Pathogen-free 6-8-week-old female immunocompetent hairless mice, crl:SKH1[hrhr]br, non-pedigreed from an albino background (20-25 gram) (Charles River Laboratories, Wilmington, MA) were studied based on previous experience with experimental pneumococcal meningitis (8). Experiments were performed in accordance local IACUC guidelines.

Anidulafungin and voriconazole (Pfizer Pharmaceuticals, Inc., New York, NY) and amphotericin B deoxycholate (X-Gen Pharmaceuticals Inc., Big Flats, NY) were studied; stock solutions were prepared as previously described (1, 2). Single-dose pharmacokinetics of anidulafungin and voriconazole were determined. Blood was collected by cardiac puncture and assayed at the Fungus Testing Laboratory, University of Texas Health Science Center (San Antonio, TX) by high performance liquid chromatography (16).

5×10^5 cfu of *C. albicans* in 20µl of PBS was injected transcutaneously into the cisterna magna under anesthesia [i.p. ketamine (100mg/kg) plus xylazine (10mg/kg)]
based on the method described in previous studies (6, 8). On day 2, treatment was
initiated and continued for 8 days.

Sixteen mice were allocated to high-dose anidulafungin (10mg/kg/day intraperitoneally [i.p.]), low-dose anidulafungin (5mg/kg/day i.p.), voriconazole (60mg/kg/day orally), amphotericin B (1.5mg/kg/day i.p.), or no treatment. Beginning 3
days prior to treatment, mice to receive voriconazole were given grapefruit juice (Ocean
Spray, Inc., Lakeville-Middleboro, MA) instead of water, to inhibit voriconazole
metabolism (2, 3, 12). Ten days after infection and 24h after the last treatment, mice were
sacrificed by i.p. injection of 100mg/kg pentobarbital. Brain and one kidney were
aseptically harvested, weighed, homogenized with 2ml PBS, and quantitatively cultured
with results expressed as cfu/g of *C. albicans*. Three mice with inability to move or eat at
the time of treatment initiation were sacrificed and excluded from further study. Their
burden of *C. albicans* (mean±SD) was 5.43±0.09log_{10} cfu/g and 5.04±0.56log_{10} cfu/g in
brain and kidney, respectively.

Animals challenged with candidal inoculum were followed for up to 10 days to
assess survival using Kaplan-Meier methodology. We also compared quantitative brain
culture results between each group of mice using the Kruskal Wallis test. Considering
that death is a worse outcome than survival, missing data due to death were imputed
using the mean log_{10} cfu/gram of brain from untreated mice dying at 4 days in our
preliminary studies, as previously described (2, 7, 13). Comparisons of categorical
variables were made using Fisher’s exact test due to small sample sizes. Tests were two-
tailed with an alpha level of 0.05.
The *C. albicans* isolate studied had MIC values of 0.25, 0.03, and 0.06µg/ml for amphotericin B, anidulafungin, and voriconazole, respectively. Plasma concentrations after single dose administration of anidulafungin and voriconazole are shown in table 1. Two days after infection (day 2), all mice appeared ill (i.e., thin skin, reduced activity). Preliminary studies in seven untreated mice sacrificed on day 4 showed that the candidal burden in brain tissues (mean and 95% confidence interval) was 4.39 (4.01-4.77) log₁₀ CFU/g. Of seven untreated mice, four had fungal burdens in the kidney below the detectable level (2.1 log₁₀ CFU/g), and the renal fungal burden of the remaining 3 was 2.71 (2.26-3.16) log₁₀ CFU/g. The fungal burden in cerebrospinal fluid (CSF) was also below the detectable level of 2.1 log₁₀ CFU/ml. In a survival analysis, 11 of 16 untreated mice died within 6 days after injection of candidal inoculum. When fungal burdens (mean and 95% confidence interval) in brain tissues were evaluated in 12 untreated mice which survived through the time of sacrifice, they declined over time [4.39 (4.01-4.77) on day 4, 3.83 (3.25-4.41) on day 6, 3.92 (1.82-6.01) on day 7, and 3.49 (3.25-3.73) on day 10, respectively].

All treatments except low-dose anidulafungin (5mg/kg/day) reduced mortality of mice infected with *C. albicans*, compared with untreated controls (P < 0.05, Figure 1). Eight days after initiation of antifungal treatment, all treatments except low-dose anidulafungin also reduced the fungal load in brain tissue (Figure 2). No significant differences in fungal burdens in brain were observed between mice treated with high-dose anidulafungin (10mg/kg/day) and amphotericin B, but the fungal burden in voriconazole group was higher than that in amphotericin B or high-dose anidulafungin group (both P < 0.05). When only surviving mice were analyzed, high-dose
anidulafungin and amphotericin B (both \(P<0.001\)) as well as voriconazole \(P=0.006\), also significantly reduced fungal burden in brain, compared with untreated controls. The inclusion of voriconazole was dependent on the concomitant use of grapefruit juice to inhibit rapid voriconazole metabolism \(2, 3, 12\). Even though voriconazole levels were attainable in mice given grapefruit juice, those at 24h after single dose administration were below \(1\mu g/ml\), which might be inadequate for treatment of serious candidal infection. The median fungal burden of voriconazole group was higher than that in the amphotericin B or high-dose anidulafungin group, possibly related to rapid metabolism of voriconazole by the mice. Our study had several limitations. First, we were unable to detect the antifungal drugs studied in CSF or brain. However, anidulafungin levels in plasma were in agreement with a previous PK/PD study in mice, and were comparable to those in humans \(1, 4, 15\). Second, for analyses of comparative brain burdens of \(C. albicans\), samples missing due to death were assigned an arbitrary value, since we assumed that death was a worse outcome than survival with any amount of fungal burden \(2, 7, 13\). For the further evaluation, a less lethal model may be needed. Despite shortcomings, we believe that our data suggest a potential role of anidulafungin as an alternative choice for the treatment of candidal CNS infection. Given the safety and efficacy of anidulafungin and its novel pharmacokinetic characteristics, further investigation is warranted to assess clinical relevance.
Acknowledgment

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References


Table 1. Plasma concentrations after single administration of antifungal agents in mice

<table>
<thead>
<tr>
<th>Time after single-dose administration</th>
<th>1 hour</th>
<th>3 hours</th>
<th>6 hours</th>
<th>12 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anidulafungin 5 mg/kg i.p.</td>
<td>2.50 ± 0.06</td>
<td>1.73 ± 0.32</td>
<td>1.66 ± 0.22</td>
<td>1.18 ± 0.03</td>
<td>0.18 ± 0.32</td>
</tr>
<tr>
<td>Anidulafungin 10 mg/kg i.p.</td>
<td>4.02 ± 1.92</td>
<td>4.91 ± 0.41</td>
<td>3.18 ± 1.11</td>
<td>2.73 ± 0.14</td>
<td>1.39 ± 0.05</td>
</tr>
<tr>
<td>Voriconazole 60 mg/kg orally</td>
<td>7.41 ± 4.66</td>
<td>7.28 ± 3.33</td>
<td>9.47 ± 2.80</td>
<td>5.37 ± 4.88</td>
<td>0.16 ± 0.14</td>
</tr>
</tbody>
</table>

Pharmacokinetic profiles of anidulafungin were determined by injecting 5 mg/kg or 10 mg/kg i.p. and obtaining plasma samples at 1, 3, 6, 12, 24 hours post injection. Those of voriconazole were determined following administration of 60 mg/kg orally.

Note. Data at each time-point are average concentrations (µg/ml) ± standard deviation of 3 mice.
Figure legends

Figure 1. Cumulative mortality of mice in the different treatment groups shown using Kaplan-Meier survival curves. Treatment was initiated on day 2 and continued for 8 days. The mortality rates of each group were as follows: Amphotericin B, 6.7% (1/15); anidulafungin 10mg/kg, 18.8% (3/16); voriconazole, 25.0% (4/16); anidulafungin 5mg/kg, 62.5% (10/16); and untreated control, 68.8% (11/16). All treatments except low-dose anidulafungin (5mg/kg/day) reduced mortality compared with untreated controls (P < 0.05). No significant differences in survival were observed between mice treated with high-dose anidulafungin (10mg/kg/day), amphotericin B and voriconazole.

Abbreviations. AmB, amphotericin B deoxycholate; AND 10, anidulafungin 10mg/kg/day; VCZ, voriconazole; AND 5, anidulafungin 5mg/kg/day; Cont, untreated control.

Figure 2. Candidal burden in brains of mice according to treatment regimen. Log$_{10}$ cfu/g of *Candida albicans* in brain in the amphotericin B (black circles, n=15), anidulafungin 10mg/kg (gray triangles, n=16), anidulafungin 5mg/kg (white circles, n=16), voriconazole (black squares, n=16), and control (gray diamonds, n=16) arms are shown. Bars represent median values. A value of 4.39 log$_{10}$cfu/g was assigned to mice dying prior to end of the 10-day follow-up (2, 7, 13). Compared with untreated controls (median, 4.39 log$_{10}$cfu/g; interquartile range, 3.69-4.39), amphotericin B (median, 2.57 log$_{10}$cfu/g; interquartile range, 2.12-2.83; P<0.001), high-dose anidulafungin (10 mg/kg/day) (median, 2.65 log$_{10}$cfu/g; interquartile range, 2.38-3.01; P<0.001) and voriconazole (median, 3.23 log$_{10}$ cfu/g; interquartile range, 2.86-3.99; P=0.002).
significantly reduced the fungal burden in brain tissues. The fungal burden in the voriconazole group was higher than that in amphotericin B or high-dose anidulafungin group (both P < 0.05). No significant difference was found between the amphotericin B and high-dose anidulafungin (10mg/kg/day) groups.

Abbreviations. AmB, amphotericin B deoxycholate; AND 10, anidulafungin 10mg/kg/day; VCZ, voriconazole; AND 5, anidulafungin 5mg/kg/day; Cont, untreated control.
Figure 1.
Figure 2.