Pentamidine Is Active In Vitro against Fusarium Species

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Fusariosis is an emerging opportunistic mycosis against which currently used antifungals have limited activity. Here, we investigated the in vitro activities of pentamidine (PNT) against 10 clinical isolates of Fusarium species (five Fusarium solani isolates and five non-F. solani isolates) by using the National Committee for Clinical Laboratory Standards microdilution method in three different media (RPMI, RPMI-2, and a yeast nitrogen base medium), disk diffusion testing, and viability dye staining. PNT had significant activities against all 10 Fusarium isolates. Non-F. solani isolates were more susceptible than F. solani isolates (P < 0.05). Additionally, PNT was fungicidal against all non-F. solani isolates, whereas it had fungistatic effects against four of the five F. solani isolates. PNT also exhibited greater activity against conidial than against hyphal development of the fungus. This fungicidal activity against non-F. solani Fusarium isolates was confirmed microscopically after staining of PNT-treated Fusarium oxysporum hyphae with the fluorescent viability dyes 5-(6)-carboxyfluorescein diacetate (CFDA) and bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC). The MICs at which 50% of the isolates were inhibited (2 μg/ml for non-F. solani isolates and 4 μg/ml for F. solani isolates) and the minimum fungicidal concentration at which 50% of the isolates were killed (8 μg/ml for non-F. solani isolates) were much lower than the PNT tissue concentrations previously reported in humans using conventional daily intravenous PNT dosing. Finally, PNT was more active against Fusarium isolates in a hypoxic environment of in vitro growth (P < 0.05). This finding may be clinically significant, because Fusarium, an angiotropic mold, causes tissue infarcts with resultant low tissue perfusion. Our findings suggest that PNT may have a role in the management of Fusarium infections. Future in vivo studies are needed to verify these in vitro findings.

Invasive fusariosis is a severe opportunistic fungal infection that is primarily encountered in patients with leukemia (7, 27, 28). Nevertheless, other profoundly immunocompromised patients, such as allogeneic bone marrow and solid-organ transplant recipients, may experience this infection (7, 27, 28). This devastating mycosis has emerged in recent years as the second most common opportunistic invasive mold infection, behind only invasive aspergillosis, in some tertiary-care cancer centers (7, 27, 28). The most common clinical presentation of fusariosis is pneumonia, which is followed by disseminated disease (27). Among the Fusarium species, Fusarium solani accounts for nearly half of the cases of invasive fusariosis in humans and is the most virulent strain according to tests in animal models (27). Fusarium oxysporum is the second most commonly encountered species, followed by Fusarium montigenum, Fusarium verticilloides, and Fusarium proliferatum (7, 17, 27, 28).

Several studies have demonstrated the limited activities of antifungal agents against Fusarium spp. both in vitro and in animal models (3, 4, 13, 25). The only somewhat active agents in vitro are amphotericin B (AMB) and the newer broad-spectrum triazoles voriconazole (VRC) and posaconazole (24, 25); nevertheless, their in vivo activity in animal models is mediocre, which is in agreement with the exceedingly high mortality rate associated with this infection in clinical practice (>80%, even with treatment) (7, 27). Irrespective of treatment, the major prognostic determinant in fusariosis cases is neutrophil recovery (D. P. Kontoyiannis, H. Hanna, R. Hachem, M. Boktour, E. Girgawy, M. Mardani, G. P. Bodey, and I. Raad, Abstr. 40th Ann. Meet. Infect. Dis. Soc. Am., abstr. 366, 2002). Thus, the introduction of new, more effective therapeutic approaches is essential to improving the prognosis of fusariosis.

Pentamidine (PNT) is an antimicrobial agent that is active against a broad spectrum of microbes, including fungi (26). Administration of PNT is a well-established approach used for prophylaxis and treatment of pneumonia caused by Pneumocystis carinii, a microorganism that was recently classified taxonomically to be a fungus (21). PNT has also been shown to have in vitro activities against a variety of fungal pathogens, such as Candida albicans, Cryptococcus neoformans, Scedosporium prolificans, and Aspergillus terreus (1, 2, 5, 20). Therefore, in this study, we evaluated the in vitro activities of PNT against 10 clinical isolates of Fusarium spp. in different culture media and various oxygen conditions and against the two development programs of these opportunistic mold species.

MATERIALS AND METHODS

Fusarium isolates. We obtained 10 clinical isolates of Fusarium spp. from the Mycology Laboratory at The University of Texas M. D. Anderson Cancer Center for testing: five isolates of the F. solani complex and five non-F. solani isolates (F.
TABLE 1. Susceptibilities of 10 clinical isolates of Fusarium spp. to antifungal agents in RPMI medium (NCCLS microdilution method M-38P)

<table>
<thead>
<tr>
<th>Agent</th>
<th>F. solani isolates (n = 5)</th>
<th>Non-F. solani isolates (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC₅₀</td>
<td>MIC₉₀</td>
</tr>
<tr>
<td>AMB</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>5FC</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>FLC</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>ITZ</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>VRC</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>CAS</td>
<td>32</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

* Units are micrograms per milliliter throughout.
* P < 0.05 for MICs of AMB against F. solani isolates versus those for non-F. solani Fusarium isolates.

Aeration, infection, and visual evaluation. The MICs of various agents were determined for F. solani, Fusarium oxysporum, F. proliferatum, and F. palidum sp. (non-F. solani Fusarium species) as described previously (23). C. tropicalis strain ATCC 2697 was used for quality control purposes in all of the experiments. F. oxysporum was prepared as described elsewhere (19). Incubation was continued at 35°C for 2 h in the dark to allow for conversion of XTT to its formazan derivatives. After shaking, the ODs at 492 and 690 nm were measured by using a microplate spectrophotometer (Powerwave X; Bio-Tek Instruments, Winooski, Vt.). The color was then assessed spectrophotometrically based on the relative OD at 492 and 690 nm, the latter of which is a reference wavelength subtracted from 492 nm.

TABLE 2. MIC-2s, MIC-9s, and MFCs of PNT against 10 clinical isolates of Fusarium spp. in RPMI and YNB media (NCCLS microdilution method M-38P)

<table>
<thead>
<tr>
<th>Fusarium sp. (no. of isolates)</th>
<th>RPMI medium</th>
<th>YNB medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC₅₀ (range, GM)</td>
<td>MIC₉₀ (range, GM)</td>
</tr>
<tr>
<td>F. solani (5)</td>
<td>4 (2–4, 3.6)</td>
<td>&gt;128 (8–128, 206.4)</td>
</tr>
<tr>
<td>Non-F. solani (5)</td>
<td>2 (1–2, 1.8)</td>
<td>8 (4–8, 6.4)</td>
</tr>
</tbody>
</table>

* GM, geometric mean.
* P < 0.001 for MIC-2s of PNT against Fusarium spp. in RPMI medium versus those in YNB medium.
* P < 0.05 for MICs of PNT against Fusarium spp. in RPMI medium versus those in YNB medium.
was performed on RPMI agar plates previously prepared using standardized conidia (10^6 conidia/ml) of each isolate was plated. After the plates were allowed to dry, a sterile 1/4-inch-thick paper disk (Schleicher & Schuell, Keene, N.H.) was placed on the agar surface and inoculated with 8.3 mg/ml in distilled water) and stored it at 70°C until use. Microtiter plates were prepared as described above and placed in chambers along with BBL gas generator envelopes (CampyPak Plus and GasPak Plus for microaerophilic and anaerobic conditions, respectively; Becton Dickinson & Co., Sparks, Md.). We then estimated the MIC-2s, MIC-0s, and MFCs of PNT against conidia to hyphae and the effects of PNT on the growth of Fusarium isolates. An analogous experiment was performed using YNB medium instead of RPMI-2. In a similar manner, we evaluated the inhibitory effect of PNT when introduced to already formed hyphae of two F. solani and two F. oxysporum isolates.

Activity of PNT against Fusarium isolates in a hypoxic chamber of in vitro growth. Microtiter plates were prepared as described above and placed in chambers along with BBL gas generator envelopes (CampyPak Plus and GasPak Plus for microaerophilic and anaerobic conditions, respectively; Becton Dickinson & Co., Sparks, Md.). We then estimated the MIC-2s, MIC-0s, and MFCs of PNT for the 10 Fusarium isolates under both microaerophilic and anaerobic conditions. We also determined the MICs and MFCs of AMB, 5FC, FLC, ITZ, VRC, and CAS against Fusarium under these hypoxic conditions.

Disk diffusion susceptibility testing. We prepared a stock solution of PNT (60 mg/ml in distilled water) and stored it at −70°C until use. Disk diffusion testing was performed on RPMI agar plates previously prepared using standardized methods. Two hundred microliters of a standardized suspension of Fusarium conidia (10^4 conidia/ml) of each isolate was plated. After the plates were allowed to dry, a sterile 1/4-inch-thick paper disk (Schleicher & Schuell, Keene, N.H.) was placed on the agar surface and inoculated with 8.3 µl of PNT, producing a final PNT concentration in each plate of 20 µg/ml. Next, plates were placed in chambers containing envelopes that produced microaerophilic or anaerobic conditions. We also determined the MICs and MFCs of AMB, 5FC, FLC, ITZ, VRC, and CAS against Fusarium under these hypoxic conditions.

Statistical analysis. The MIC-2s and MIC-0s of PNT for the 10 Fusarium isolates in RPMI medium were compared with those in YNB medium. Additionally, the MIC-2s, MIC-0s, and MFCs of PNT and the MICs and MFCs of VRC for F. solani isolates were compared with those for non-F. solani Fusarium isolates. The Mann-Whitney two-tailed t test was used to assess significant differences in the corresponding MIC-2s, MIC-0s, and MFCs. In addition, the MIC-2s and MIC-0s (as well as the radius of the zone of growth inhibition) of PNT for the 10 Fusarium isolates under aerobic conditions were compared with those under microaerophilic and anaerobic conditions. Analysis of variance was used to assess differences in the corresponding MICs, and Kruskal-Wallis one-way analysis of variance with Dunn’s test was used with the GraphPad Prism 3 software program to assess statistically significant differences (GraphPad Software, Inc., San Diego, Calif.). The 50% (EC_{50}) and 90% (EC_{90}) effective concentrations of PNT were determined by fitting XTT time-kill data to a sigmoidal dose-response model by using the following four-parameter logistic equation:

\[ y = \frac{EC_{\text{max}}}{1 + 10 \log \left( \frac{C}{EC_{\text{min}}} \right)} \]

where EC_{\text{min}} represents the lowest PNT concentration producing inhibitory

### Table 3. XTT-based pharmacodynamic analysis of PNT activities against two Fusarium spp. and two developmental programs in two culture media*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Species</th>
<th>Developmental program</th>
<th>PNT conc (µg/ml)</th>
<th>EC_{50}</th>
<th>EC_{90}</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI-2</td>
<td>F. solani</td>
<td>Conidial</td>
<td>3.9</td>
<td>29.7</td>
<td>&gt;128</td>
</tr>
<tr>
<td>RPMI-2</td>
<td>F. solani</td>
<td>Hyphal</td>
<td>53.1</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>RPMI-2</td>
<td>F. oxysporum</td>
<td>Conidial</td>
<td>1.6</td>
<td>5.8</td>
<td>&gt;128</td>
</tr>
<tr>
<td>RPMI-2</td>
<td>F. oxysporum</td>
<td>Hyphal</td>
<td>30.3</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>YNB</td>
<td>F. solani</td>
<td>Conidial</td>
<td>1.4</td>
<td>8.6</td>
<td>&gt;128</td>
</tr>
<tr>
<td>YNB</td>
<td>F. solani</td>
<td>Hyphal</td>
<td>50.5</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>YNB</td>
<td>F. oxysporum</td>
<td>Conidial</td>
<td>0.5</td>
<td>1.57</td>
<td>&gt;128</td>
</tr>
<tr>
<td>YNB</td>
<td>F. oxysporum</td>
<td>Hyphal</td>
<td>17.8</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

*The pharmacodynamic analysis of PNT against conidia was based on three isolates each of F. solani and F. oxysporum, whereas that of PNT against hyphae was based on two isolates each of F. solani and F. oxysporum.
activity, EC_{max} represents the maximal PNT concentration that achieves maximal inhibitory activity, and Hill is the variable slope factor, or Hill coefficient of the dose-response curve.

RESULTS

Susceptibility of Fusarium spp. to antifungal agents and PNT. We found that AMB, VRC, and PNT were the only active agents against the Fusarium isolates, whereas 5FC, FLC, ITZ, and CAS had no activity. The MICs and MFCs of AMB, 5FC, FLC, ITZ, VRC, and CAS are listed in Table 1. Specifically, AMB had comparable fungicidal activities against the solani and non-F. solani Fusarium isolates. Also, VRC was fungicidal against the non-F. solani Fusarium isolates, whereas it was fungistatic against the F. solani isolates. In comparison, ITZ had very limited fungistatic activity against the non-solani Fusarium isolates. The MIC-2s, MIC-0s, and MFCs of PNT against Fusarium isolates in RPMI and YNB media are shown in Table 2. PNT showed activity against all 10 of the Fusarium isolates tested. However, the MICs of PNT against non-F. solani Fusarium isolates were lower than those against F. solani isolates (P < 0.05). In addition, based on the MFC/MIC ratios, PNT was fungicidal against all five non-F. solani Fusarium isolates and one F. solani isolate and was fungistatic against the remaining four F. solani isolates. The PNT MICs were identi-
cal in the RPMI and RPMI-2 media (data for RPMI-2 medium not shown), whereas they were significantly lower in the YNB medium \((P < 0.001)\). In contrast, the MFCs of PNT in the RPMI and YNB media did not differ significantly.

In agreement with the NCCLS results, use of the disk diffusion method showed that the radii of the zones of growth inhibition of \(F.\ solani\) isolates caused by PNT were smaller than those of non-\(F.\ solani\) Fusarium isolates. The mean radii for the \(F.\ solani\) and non-\(F.\ solani\) isolates were 5.867 mm (standard deviation [SD], ± 2.694 mm) and 14.660 mm (SD, ± 1.65 mm), respectively \((P < 0.05)\).

Comparison of the visual and spectrophotometric readings of the NCCLS method for \(Fusarium\) spp. The MIC-2 and MIC-0 endpoints of PNT as measured spectrophotometrically using the XTT colorimetric assay were comparable to the respective MICs estimated visually using the NCCLS method. More specifically, by using the MIC-2 endpoint, in 8 of the 10 \(Fusarium\) isolates the visual and spectrophotometric MIC-2s were identical, while in 2 isolates the values differed for one drug dilution. By using the MIC-0 endpoint, 9 of the 10 isolates had identical visual and spectrophotometric MIC-0s, whereas in 1 isolate those values differed for one drug dilution. Furthermore, in 9 of the 10 isolates tested, the MICs of AMB were identical when assessed both visually and spectrophotometrically.

**XTT-based pharmacodynamic analysis of PNT activity.** We generated dose-response curves of PNT and calculated the effective PNT concentrations that caused 50\% (EC\(_{50}\)) and 90\% (EC\(_{90}\)) reductions in XTT absorbance compared with drug-free XTT absorbance at 48 h after the addition of PNT. The EC\(_{50}\) and EC\(_{90}\) of PNT against the two developmental programs of growth of \(F.\ solani\) and \(F.\ oxysporum\) in two culture media are summarized in Table 3. Again, PNT showed preferential activity against the conidial development program of \(Fusarium\) spp. compared with the hyphal development program (Fig. 1). Also, in agreement with the MIC and MFC

<table>
<thead>
<tr>
<th>Fusarium sp. (no. of isolates)</th>
<th>MIC-2(_a) (range, GM(_a))</th>
<th>MIC-0(_a) (range, GM(_a))</th>
<th>MIC-2(_b) (range, GM(_b))</th>
<th>MIC-0(_b) (range, GM(_b))</th>
<th>MIC-2(_c) (range, GM(_c))</th>
<th>MIC-0(_c) (range, GM(_c))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F.\ solani) (5)</td>
<td>4 (2–4, 3.6)</td>
<td>32 (8–32, 24)</td>
<td>2 (1–2, 1.8)</td>
<td>16 (8–16, 12.8)</td>
<td>1 (1–2, 1.2)</td>
<td>8 (4–16, 8.8)</td>
</tr>
<tr>
<td>Non-(F.\ solani) (5)</td>
<td>2 (1–2, 1.8)</td>
<td>8 (4–8, 6.4)</td>
<td>0.5 (0.5–1, 0.6)</td>
<td>2 (1–2, 1.6)</td>
<td>0.250 (0.125–0.500, 0.220)</td>
<td>1.0 (0.5–1.0, 0.9)</td>
</tr>
</tbody>
</table>

\(a\) GM, geometric mean.  
\(b\) \(P < 0.05\) for MIC-2s of PNT against \(Fusarium\) spp. under aerobic conditions versus those under microaerophilic conditions.  
\(c\) \(P < 0.001\) for MIC-2s of PNT against \(Fusarium\) spp. under aerobic conditions versus those under anaerobic conditions.  
\(d\) \(P < 0.05\) for MIC-0s of PNT against \(Fusarium\) spp. under aerobic conditions versus those under anaerobic conditions.  
\(e\) Units are micrograms per milliliter throughout.
results, the EC50s and EC90s of PNT were lower against *F. oxysporum* than against *F. solani* and were lower in YNB medium than in RPMI-2 medium.

**CFDA and DiBAC staining.** After staining with CFDA and DiBAC, we found that PNT killed apical and subapical compartments of viable *F. oxysporum* hyphae at a concentration equal to the MFC against the *F. oxysporum* isolate used. This fungicidal activity was more pronounced when hyphae were exposed to higher PNT concentrations. Figure 2D and E show the CFDA and DiBAC staining in a *F. oxysporum* isolate after challenge with PNT (at a concentration equal to the MFC and four times the MFC, respectively). Interestingly, AMB did not kill all of the hyphal elements at a concentration equal to the MFC (Fig. 2C). In the *F. solani* isolate tested, PNT had no fungicidal effects when added at the MIC-0; however, at a much higher concentration (equal to eight times the MIC-0 against that isolate), compartments of dead hyphae were seen (Fig. 3).

**Activity of PNT under hypoxic conditions.** The MIC-2s and MIC-0s of PNT were lower under microaerophilic and anaerobic conditions than under normal oxygen concentrations (Table 4). The MFCs of PNT against all of the *Fusarium* isolates did not differ from those estimated under normal oxygen conditions. In contrast, the low-oxygen environment did not affect the MICs and MFCs of AMB, FLC, 5FC, ITZ, VRC, and CAS. By using the disk diffusion method, we found that the radius of the zone of growth inhibition caused by PNT was larger under microaerophilic and anaerobic conditions than under normal oxygen levels (*P* < 0.05) (Table 5) (Fig. 4). In contrast, the zone of growth inhibition caused by AMB was not affected by the different oxygen conditions, as the geometric means of the radii of the zones in a normal oxygen environment and under microaerophilic and anaerobic conditions were 1.83, 1.73, and 1.92 mm, respectively (*P* value was not significant).

**DISCUSSION**

We investigated the in vitro activities of PNT against 10 clinical isolates of *Fusarium* spp. and against the different developmental programs of the fungus by using independent susceptibility methods, different media, and various environmental conditions. The susceptibilities of *Fusarium* isolates to the other antifungals tested were in agreement with those reported in previous studies (4, 13, 25). Specifically, AMB and VRC were the only other active agents. However, the MIC50s and MFC50s of AMB were higher than the previously reported tissue concentration of AMB in the lungs (0.5 μg/ml) when conventional intravenous AMB doses (1.0 to 1.5 mg/kg of body weight/day) were used (15). Furthermore, the activity of VRC was variable and depended on the *Fusarium* species tested. Although VRC was fungicidal against the non- *F. solani* *Fusarium* isolates, it had moderate fungistatic effects against the *F. solani* isolates. As previously reported, CASES, FLC, and ITZ had no activity against the *Fusarium* isolates that were tested (4, 13, 25).

Here, we demonstrate that PNT has notable in vitro activity against *Fusarium* spp. PNT had higher activity and was fungicidal against the non-*F. solani* *Fusarium* isolates, while it had fungistatic effects against the majority of the *F. solani* isolates. Several studies have shown that *F. solani* is, in general, more resistant to antifungals than are non-*F. solani* *Fusarium* spp. (4, 13, 25, 27). In our study, this higher resistance of *F. solani* isolates versus non-*F. solani* *Fusarium* isolates under aerobic conditions versus those under anaerobic conditions.

### Table 5. Disk diffusion susceptibility testing of PNT activities against *Fusarium* spp. under different oxygen conditions

<table>
<thead>
<tr>
<th>Fusarium sp. (no. of isolates)</th>
<th>Aerobic conditions</th>
<th>Microaerophilic conditions</th>
<th>Anaerobic conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>Fusarium</em> spp. (10)</td>
<td>10.267 ± 3.829a</td>
<td>17.283 ± 3.695</td>
<td>20.783 ± 5.168c</td>
</tr>
<tr>
<td><em>F. solani</em> (5)</td>
<td>5.867 ± 2.694c</td>
<td>13.2 ± 3.532</td>
<td>16.6 ± 3.227c</td>
</tr>
<tr>
<td>Non-<em>F. solani</em> (5)</td>
<td>14.66 ± 1.65c</td>
<td>21.367 ± 2.162</td>
<td>24.967 ± 2.434f</td>
</tr>
</tbody>
</table>

* GM, geometric mean.
* Each disk contained 8.3 μl of PNT (from a stock solution of 60 mg/ml).
* *P* < 0.05 for the radii of the zones of growth inhibition caused by PNT against all isolates under aerobic conditions versus those under anaerobic conditions.
* *P* < 0.05 for the radii of the zones of growth inhibition caused by PNT against *F. solani* isolates versus non-*F. solani* *Fusarium* isolates in room air.
* *P* < 0.05 for the radii of the zones of growth inhibition caused by PNT against *F. solani* isolates versus non-*F. solani* *Fusarium* isolates under aerobic conditions versus those under anaerobic conditions.
FIG. 4. Effects of PNT against an \textit{F. solani} isolate (isolate no. 2) under different oxygen conditions, i.e., natural oxygen conditions (A), microaerophilic conditions (B), and anaerobic conditions (C), as seen in disk diffusion susceptibility testing (each disk contained 8.3 μl of PNT from a 60-mg/ml stock solution).

of the isolate used. Interestingly, PNT had similar effects against \textit{F. solani}, although at much higher concentrations.

The limited number of pharmacokinetic studies using tissue samples obtained from PNT-treated humans upon autopsy have shown high concentrations of PNT in tissues when the drug is administered at a conventional therapeutic dose (4 mg/kg/day intravenously) (6). Blood PNT concentrations have been reported to range from 0.5 to 3.2 μg/ml (6). However, much higher levels are typically seen in tissues, with concentrations of up to 56 μg/g in the lungs, 123 μg/g in the kidneys, 300 μg/g in the liver, and 368 μg/g in the spleen (6). When compared with these reported tissue concentrations of PNT, the MIC<sub>90</sub>, MIC<sub>90</sub>, and MFC<sub>50</sub> of PNT in our study are much lower.

By using XTT colorimetric assay, we demonstrated preferentially increased activity of PNT against the conidial versus the hyphal developmental state of the fungus. This observation is consistent with the results of several studies showing that the hyphal forms of filamentous fungi are inherently more resistant to antifungal agents than are the conidial forms (12, 14). Guarro et al. (12) showed that the MICs and MFCs of AMB, 5FC, FLC, miconazole, ketoconazole, and ITZ against \textit{Cladosporium}, \textit{Paeclomyces}, \textit{Scopulariopsis}, and \textit{Cladophialophora} species were significantly (2- to 512-fold) higher when these antifungals were tested against already formed hyphae than when the antifungals were tested against the conidial forms of these molds. Similar results have been observed for \textit{Aspergillus} species (14).

We found that the EC<sub>50</sub> and EC<sub>90</sub> of PNT against the conidial forms of \textit{Fusarium} spp. were significantly lower than the PNT concentrations that can be achieved in tissues (6). The respective PNT values against the hyphal forms of the \textit{Fusarium} spp. were appreciably higher, yet the EC<sub>50</sub> were again lower than the achievable PNT concentrations. Conidial colonization is the first step in the pathogenesis of invasive fusariosis. In immunocompetent hosts, conidia are inhaled into the lungs in large numbers and are efficiently phagocytosed there by the resident macrophages (9). However, in immunocompromised patients with defects in macrophage function, some conidia escape phagocytosis, germinate to hyphae, and establish an invasive infection in the setting of prolonged neutropenia (9). Thus, our findings imply that PNT could be more promising for prophylaxis against \textit{Fusarium} infections.

Even though PNT has been used clinically for more than 2 decades, the mechanisms of its action have not been definitively established. Several mechanisms have been proposed, such as inhibition of DNA, RNA, phospholipid, and protein synthesis (26). Interestingly, we found that PNT was more effective against \textit{Fusarium} spp. under low-oxygen conditions, as shown using two independent methods: the NCCLS microdilution method and disk diffusion susceptibility testing. Even though this difference may reflect less vigorous growth of the fungus in an environment of low-oxygen tension, these data may indicate that PNT actually has preferentially greater efficacy against \textit{Fusarium} spp. under such oxygen conditions, as the hypoxic environment did not alter the activities of the other antifungals tested. Whether this observation reflects the conversion of PNT to an active prodrug in the setting of low-oxygen tension in a mechanism reminiscent of the activity of metronidazole against bacteria (10) remains to be determined. However, these results have promise for further in vivo testing of PNT against fusariosis. Like \textit{Aspergillus}, \textit{Fusarium} is an angiotropic mold that invades vessels, resulting in tissue infarcts, low tissue perfusion, and suboptimal efficacy of antifungals (P. J. Patterson, E. M. Johnson, S. Ainscough, H. G. Prentice, M. Potter, and C. C. Kibbler, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1328, 2000).

Given the establishment of a semianaerobic environment as a result of invasive fusariosis, this in vitro enhanced activity of PNT under hypoxic conditions may be of clinical importance.

In conclusion, this report is the first description of the in vitro activities of PNT against a variety of pathogenic \textit{Fusarium} spp. Our findings suggest that the role of PNT in the treatment of \textit{Fusarium} infections should be explored further. More studies using appropriate animal models and possibly clinical studies will be needed to elucidate the potential of this drug for prophylaxis or treatment of \textit{Fusarium} infections in immuno-compromised patients. Such studies are currently under way in our laboratory.

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REFERENCES


