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In Vitro Antifungal Activity of Pneumocandin L-743,872 against a Variety of Clinically Important Molds

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The in vitro activity of the new antifungal drug pneumocandin L-743,872 against 55 isolates of clinically important molds was examined by an adapted macrobroth dilution method for yeasts. Pneumocandin L-743,872 exhibited in vitro antifungal activity against *Alternaria* sp., *Aspergillus flavus*, *Aspergillus fumigatus*, *Curvularia lunata*, *Exophiala jeanselmei*, *Fonsecaea pedrosoi*, *Paecilomyces variotii*, and *Scedosporium apiospermum*. The drug appeared to lack significant in vitro inhibitory activity against *Fusarium oxysporum*, *Fusarium solani*, *Rhizopus arrhizus*, *Paecilomyces lilacinus*, and *Scedosporium prolificans*.

The incidence of fungal infections in immunocompromised hosts has increased significantly in the last 20 years (10). Although the number of serious infections caused by opportunistic molds is small compared with the number caused by yeasts, they can be extremely difficult to manage. There are few specific guidelines and few available drugs for treatment of these infections. Evaluation of new antifungal agents through in vitro susceptibility testing can help establish guidelines for the potential clinical application of new therapies.

L-743,872 represents a new class of antifungal drugs that appear to target 1,3-β-glucan synthase and its function in the synthesis of the fungal cell wall. This drug is a water-soluble pneumocandin with potent activity against *Candida* species, including fluconazole-resistant isolates (6) and *Torulopsis* (*Candida*) *glabrata* (9). It has been shown previously that this compound has potent in vitro activity against *Aspergillus fumigatus* (2). In vivo murine studies have also confirmed L-743,872 to be a potent antifungal drug for the treatment of histoplasmosis (4), aspergillosis (8), and disseminated candidiasis (3). However, few data are available on its activity against a broader spectrum of clinically important molds. Recent progress in the development of guidelines for antifungal susceptibility testing of yeasts can be applied to in vitro susceptibility testing of various filamentous fungi (1, 7).

In this study, the effectiveness of L-743,872 against 55 isolates of clinically important molds was examined by adapting a macrobroth dilution method used for yeasts (5).

Materials and methods. (i) Test organisms. Fifty-five clinical isolates of filamentous fungi were evaluated in this study. The fungal isolates included one isolate of *Alternaria* sp. (150.95), eight isolates of *Aspergillus flavus* (112.96, 127.89, 234.86, 141.88, 107.96, 101.91, 156.90, and 103.86), eight isolates of *Aspergillus fumigatus* (168.95, 165.86, 153.90, 104.96, 166.95, 148.90, 101.90, and 138.89), four isolates of *Curvularia lunata* (328.86, 141.90, 110.90, and 146.90), two isolates of *Exophiala jeanselmei* var. *lecanii-corni* (308.96 and 139.90), four isolates of *Fonsecaea pedrosoi* (109.95, 106.95, 142.86, and 158.90), five isolates of *Fusarium oxysporum* (165.89, 164.89, 290.86, 161.86, and 114.86), five isolates of *Fusarium solani* (127.91, 152.89, 101.95, 145.95, and 130.88), five isolates of *Paecilomyces lilacinus* (137.90, 135.90, 106.96, 105.91, and 118.96), two isolates of *Paecilomyces variotii* (112.95 and 136.95), five isolates of *Rhizopus arrhizus* (117.89, 170.89, 182.88, 127.88, and 181.88), four isolates of *Scedosporium apiospermum* (140.88, 332.86, 287.86, and 128.91), and two isolates of *Scedosporium prolificans* (114.87 and DT229). *Aspergillus flavus* and *Aspergillus fumigatus* isolates were tested three times; all other isolates were tested twice, on different days, except for *Alternaria* sp. and *E. jeanselmei* isolates, which were each tested once.

(ii) Antifungal agent. The pneumocandin L-743,872 was provided by Merck as a pure powder. A stock solution with a concentration of 10,000 μg/ml was prepared in sterile distilled water.

(iii) Medium. Antifungal susceptibility testing was performed in glutamine-supplemented, sodium bicarbonate-free RPMI-1640 medium (Sigma) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS).

(iv) Broth macrodilution method. Macrodilution broth assays were performed as previously described (7). Drug dilutions were prepared at 10 times the strength of the final concentrations by an additive drug dilution schema designed to reduce pipetting errors (5). The 10× drug dilutions were dispensed as 0.1-ml volumes into sterile polystyrene tubes (12 by 75 mm; Falcon 2054; Becton Dickinson) and stored at −20°C until used. Stored drug dilutions were used within 2 weeks. Fungi were grown on potato dextrose agar at 30°C and subcultured twice to ensure viability. After adequate sporulation occurred (4 to 12 days), spores were harvested by flooding colonies with a sterile solution consisting of 0.85% NaCl and 0.05% Tween 80 in distilled water. Inoculum suspensions of 10^6 CFU/ml were prepared by a hemocytometric procedure, diluted 1:100 in NaCl-Tween solution, and then diluted 1:20 in RPMI 1640 medium to obtain an inoculum of approximately 0.5×10^3 to 2.5×10^3 CFU/ml. Inoculum size for all tests was verified by plating 10 μl of each inoculum onto a Sabouraud dextrose agar plate, incubating the plates at 30°C, and enumerating the resultant growth. Each MIC tube was inoculated with 0.9 ml of the fungal inoculum; this step yielded the final range of concentrations (0.09 to 100 μg/ml). All tubes were incubated without agitation at 30°C, and readings were taken when good growth in the control tube was evident. The MIC was defined as the lowest drug concentration yielding turbidity (noted visually) less than or equal to that corresponding to 80% inhibition compared with the growth control tube (7).

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TABLE 1. In vitro activity of L-743,872 against 55 clinically important molds

Species (no. of isolates tested)	MIC (μg/ml)		Inoculum (CFU/ml)
	Geometric mean[a]	Range	
Alternaria sp. (1)	≤0.09	≤0.09	1.2×10^3
Aspergillus flavus (8)	0.20	≤0.09–3.12	0.6×10^3–2.0×10^3
Aspergillus fumigatus (8)	≤0.09	≤0.09	1.1×10^3–2.8×10^3
Curvularia lunata (4)	0.38	≤0.09–0.78	0.8×10^3–1.3×10^3
Exophiala jeanselmei (2)	1.10[b]	0.39–3.12	2.2×10^3
Fonsecaea pedrosoi (4)	0.13[c]	≤0.09–0.19	0.3×10^3–2.1×10^3
Fusarium oxysporum (5)	75.78	50–>100	0.5×10^3–2.2×10^3
Fusarium solani (5)	59.46	50–>100	0.6×10^3–1.1×10^3
Paecilomyces lilacinus (5)	49.98	3.12–>100	1.1×10^3–2.6×10^3
Paecilomyces variotii (2)	≤0.09	≤0.09	1.5×10^3–2.3×10^3
Rhizopus arrhizus (5)	>100[d]	>100	0.6×10^3–1.3×10^3
Scedosporium apiospermum (4)	0.38	0.19–0.78	0.2×10^3–0.8×10^3
Scedosporium prolificans (2)	8.83	6.25–12.5	0.8×10^3–1.1×10^3

[a] Unless otherwise noted, incubation was for 72 h at 30°C.
[b] Incubation was for 144 h at 30°C.
[c] Incubation was for 120 h at 30°C.
[d] Incubation was for 24 h at 30°C.

(v) Analysis of the results. Geometric-means MICs were determined for each of the fungal groups.

Results and discussion. Most isolates produced adequate growth within 72 h. Isolates of *R. arrhizus* were interpreted at 24 h. Isolates of *Fonsecaea pedrosoi* and *E. jeanselmei* were interpreted at 120 and 144 h, respectively. The MIC range and the geometric-mean MIC of L-743,872 for each fungal species are summarized in Table 1. No variation in MIC was found in any of the replicate tests. The geometric-mean MICs of L-743,872 were as follows: 0.20 and ≤0.09 μg/ml for eight isolates each of *Aspergillus flavus* and *Aspergillus fumigatus*, respectively; 0.38 μg/ml for four isolates of *C. lunata*; 1.10 μg/ml for two isolates of *E. jeanselmei*; 0.13 μg/ml for four isolates of *Fonsecaea pedrosoi*; 75.78 and 59.49 μg/ml for five isolates each of *Fusarium oxysporum* and *Fusarium solani*, respectively; 49.98 and ≤0.09 μg/ml for five and two isolates of *P. lilacinus* and *P. variotii*, respectively; >100 μg/ml for five isolates of *R. arrhizus*; and 0.38 and 8.83 μg/ml for four and two isolates each of *S. apiospermum* and *S. prolificans*, respectively. *Alternaria* sp. showed an L-743,872 MIC of ≤0.09.

There is continued interest in identifying methods that can be standardized for in vitro antifungal susceptibility testing of molds (1, 7). We have detailed the method used in this study, including determination of end points, so that our results can be compared with those obtained by other methods. The methodology used in this study to prepare the inocula of filamentous fungi yielded between 0.2×10^3 and 2.8×10^3 CFU/ml. Only three inocula had sizes outside the range recommended by the National Committee for Clinical Laboratory Standards in the performance of antifungal susceptibility testing of yeasts (5): one isolate of *S. apiospermum* (0.2×10^3 CFU/ml), one isolate of *Aspergillus fumigatus* (2.8×10^3 CFU/ml), and one isolate of *P. lilacinus* (2.6×10^3 CFU/ml). As found by Pujol et al. (7), the widest variation occurred with isolates of *Fusarium* spp. (Table 1).

Clear MIC 80% end points were observed for *Alternaria* sp., *Aspergillus fumigatus*, *E. jeanselmei*, *Fonsecaea pedrosoi*, *Fusarium oxysporum*, *Fusarium solani*, *P. lilacinus*, *P. variotii*, *R. arrhizus*, *S. apiospermum*, and *S. prolificans*. Persistent partial inhibition (trailing end points) near the 80% level made end point interpretation less obvious for *Aspergillus flavus* and *C. lunata*. However, antifungal activity against these species was clearly present. Further testing of in vivo efficacy may clarify the significance of trailing end points for these species (2).

Our results showed that L-743,872 has in vitro activity against *Alternaria* sp., *Aspergillus flavus*, *Aspergillus fumigatus*, *C. lunata*, *E. jeanselmei*, *Fonsecaea pedrosoi*, *P. variotii*, and *S. apiospermum*. In contrast, the drug appears to lack strong in vitro inhibitory activity against *Fusarium oxysporum*, *Fusarium solani*, *R. arrhizus*, *P. lilacinus*, and *S. prolificans.*

In conclusion, these results suggest that L-743,872 should be considered for study in the treatment of certain mold infections. Clinical experience will be needed to confirm the efficacy of L-743,872 in the treatment of infections caused by molds that are inhibited in vitro by L-743,872. Further in vitro testing involving additional filamentous fungi is also warranted.

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