Vol. 53, No. 4

Activity of Anidulafungin in a Murine Model of *Candida krusei* Infection: Evaluation of Mortality and Disease Burden by Quantitative Tissue Cultures and Measurement of Serum (1,3)-β-D-Glucan Levels[▽]

Luis Ostrosky-Zeichner,[1]* Victor L. Paetznick,[1] Jose Rodriguez,[1] Enuo Chen,[1] and Daniel J. Sheehan[2]

Laboratory of Mycology Research, University of Texas Medical School at Houston, Houston, Texas,[1] and Pfizer, Inc., New York, New York[2]

Experience with anidulafungin against *Candida krusei* is limited. Immunosuppressed mice were injected with 1.3×10^7 to 1.5×10^7 CFU of *C. krusei*. Animals were treated with saline, 40 mg/kg fluconazole, 1 mg/kg amphotericin B, or 10 and 20 mg/kg anidulafungin for 5 days. Anidulafungin improved survival and significantly reduced the number of CFU/g in kidneys and serum β-glucan levels.

Non-*albicans Candida* species are increasing in incidence as causes of invasive candidiasis in the United States and worldwide (11). This is particularly important as *Candida krusei* has intrinsic resistance to azoles, and there are reports of reduced susceptibility to amphotericin B (AMB) (9, 16, 17). Recent research has shown that even minor delays in appropriate antifungal therapy are associated with increased morbidity and mortality and that it is difficult to predict non-*albicans Candida* species infection reliably (2, 3).

Echinocandins are novel antifungals that have activity in vitro activity against *C. krusei* and other non-*albicans Candida* species (19, 21). Proof in an animal model that *C. krusei* can be treated with anidulafungin (AFG) would be a valuable contribution to the understanding of the drug, especially since clinical trials have limited numbers of patients with infection by these species. Our objective was to measure the efficacy of AFG in terms of survival and organism burden as measured by quantitative tissue cultures and serum (1,3)-β-D-glucan (BG) levels in a murine model of *C. krusei* infection. BG levels are emerging as possible surrogate marker to evaluate the response to treatment of fungal infections (12).

(This study was presented as abstract M-1840 at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2007 [14].)

Animals. Male CF-1 mice (Harlan Sprague-Dawley) weighing 20 to 30 g were used in this study. This study was reviewed and approved by the University of Texas Health Science Center Animal Welfare Committee.

Isolate and inoculum. *C. krusei* strain LMR 39-14, a bloodstream isolate from a clinical isolate collection, was used for all experiments. The isolate species was confirmed by the API 32 method (Biomerieux), and antifungal susceptibility testing was carried out by the CLSI M27-A3 microdilution method (1). The MICs for fluconazole (FLU), AMB, and AFG were >64, 1.0, and 0.06 μg/ml, respectively. Animals were inoculated by

intravenous tail injection with 1.3×10^7 to 1.5×107 CFU per animal. Inocula were confirmed by serial plating after each experiment.

Immunosuppression. Animals were immunosuppressed by a single dose of 200 mg/kg of body weight fluorouracil (5-FU) intraperitoneally (i.p.) 3 days prior to inoculation and a single dose of 4 mg/kg of body weight dexamethasone subcutaneously on the day of inoculation.

Experimental interventions. Twenty-five animals per group were assigned to the following treatment regimens: (i) i.p. normal saline every 24 h (q24h) (placebo) for 5 days, (ii) 10 mg/kg i.p. AFG q24h for 5 days, (iii) 20 mg/kg i.p. AFG q24h for 5 days, (iv) 40 mg/kg i.p. FLU q24h for 5 days, and (v) 1 mg/kg i.p. AMB q24h for 5 days. AFG doses were based on previous pharmacodynamic studies (4, 20). The experiment was run in duplicate.

Evaluations. Fifteen animals from each group were observed for survival. Five animals from each group were sacrificed on day 5 postinoculation and five animals on day 10 postinoculation to harvest the kidneys and blood. Quantitative tissue cultures were performed by homogenization of both kidneys, serial dilutions, and plating on Sabouraud dextrose agar. Plates were incubated for 48 h, and the numbers of CFU per gram of tissue were recorded and calculated. The level of BG in serum was measured using the Fungitell kit following the manufacturer's instructions (Fungitell package insert, 2007; Associates of Cape Cod, Falmouth, MA). The detection thresholds were 40 CFU/g of tissue for quantitative cultures and 31 pg/ml for BG (according to the Fungitell package insert).

Statistical analysis. As results were similar between the duplicate runs, data were pooled for the final analysis. Survival curves were estimated using the Kaplan-Meier product limit method and compared using the log-rank test. Log-transformed CFU counts and BG levels were compared by analysis of variance and the Kruskal-Wallis method, respectively. Findings were considered statistically significant if P was <0.05.

As shown in Fig. 1, survival was statistically improved for both AFG groups compared to the saline, FLU, or AMB groups. There were no significant differences among the two AFG doses, and likewise there were no significant differences

* Corresponding author. Mailing address: Division of Infectious Diseases, University of Texas Medical School at Houston, 6431 Fannin, MSB 2.112, Houston, TX 77030. Phone: (713) 500-6733. Fax: (713) 500-5495. E-mail: Luis.Ostrosky-Zeichner@uth.tmc.edu.
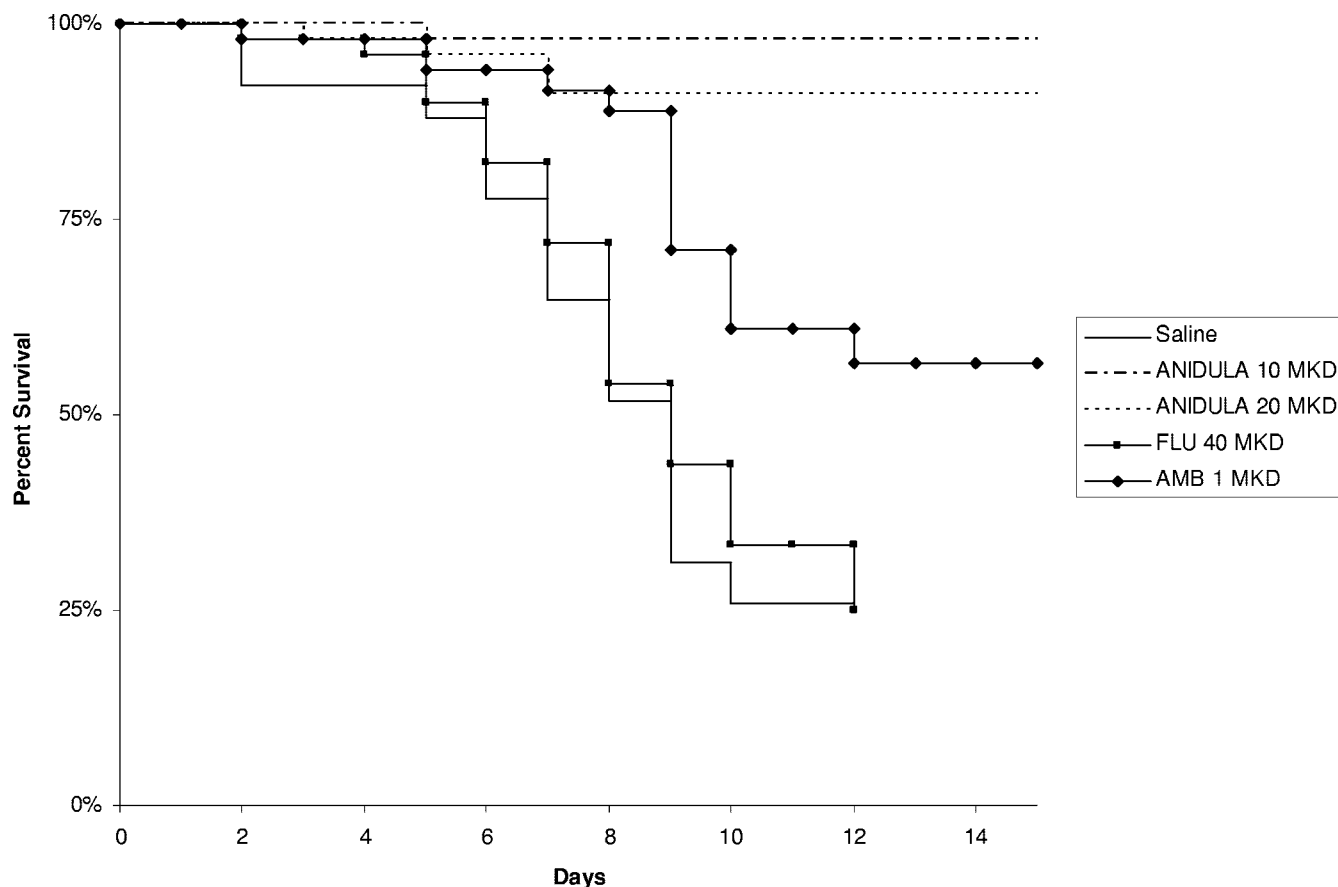
FIG. 1. Kaplan-Meier plot of survival estimates for immunocompromised CF-1 mice infected with 1.3×10^7 to 1.5×10^7 CFU of *C. krusei* by intravenous tail injection. Treatment group abbreviations ($n = 30$): Saline, i.p. normal aline solution; Anidula 10 MKD, 10 mg/kg/day i.p. AFG; Anidula 20 MKD, 20 mg/kg/day i.p. AFG; Flu 40 MKD, 40 mg/kg/day i.p. FLU; AMB 1 MKD, 1 mg/kg/day i.p. AMB. Both AFG doses statistically improved survival ($P < 0.05$) compared to placebo, AMB, or FLU. AMB statistically improved survival ($P < 0.05$) compared to placebo and FLU.

between FLU and saline. AMB significantly prolonged survival compared to saline and FLU.

As shown in Table 1, both doses of AFG produced a statistically significant ~2-log reduction of kidney CFU/g compared to saline and FLU on day 5 and an ~4-log reduction by day 10. No statistically significant reductions were seen for FLU or

TABLE 1. Tissue burden in kidneys and BG levels in serum

Treatment group[a]	Day 5[b]		Day 10[b]	
	Mean ± SD tissue burden (log₁₀ CFU/g)	Mean ± SD serum BG level (pg/ml)	Mean ± SD tissue burden (log₁₀ CFU/g)	Mean ± SD serum BG level (pg/ml)
Uninfected	ND	52 ± 30	ND	44 ± 72
Saline	6.66 ± 0.30	1,942 ± 574	5.22 ± 2.17	1,670 ± 962
FLU	7.01 ± 0.56	2,714 ± 2,636	4.77 ± 1.74	1,292 ± 734
AMB	5.95 ± 0.47	2,559 ± 2,872	4.96 ± 0.66	1,742 ± 1,108
AFG10	4.06 ± 0.38*†‡	256 ± 144*†‡	0.96 ± 1.54*†‡	624 ± 497*†‡
AFG20	3.97 ± 0.34*†‡	351 ± 264*†‡	1.59 ± 1.68*†‡	693 ± 816*‡

[a] The treatment regimens were as follows: Saline, normal saline solution q24h (placebo) for 5 days; FLU, 40 mg/kg i.p. FLU q24h for 5 days; AMB, 1 mg/kg i.p. AMB q24h for 5 days; AFG10, 10 mg/kg i.p. AFG q24h for 5 days; and AFG20, 20 mg/kg i.p. q24h for 5 days.
[b] Log₁₀-transformed CFU were compared by analysis of variance, and BG levels were compared by the Kruskal-Wallis method. ND, not determined. *, $P < 0.05$ compared to saline; †, $P < 0.05$ compared to FLU; ‡, $P < 0.05$ compared to saline.

AMB compared to saline, and no differences were seen between the two doses of AFG either. BG levels significantly decreased with both AFG doses compared to saline, FLU, and AMB on days 5 and 10. There were no significant differences between the two doses or between the levels found for each dose on day 5 and day 10.

AFG showed antifungal activity in this murine model of *C. krusei* infection, as evidenced by improved survival and reduced organism burden by quantitative tissue cultures and serum BG.

AFG shows good in vitro activity against *C. krusei*. ARTEMIS, a global surveillance program (17), reported a MIC₉₀ of 0.06 μg/ml for 121 isolates of this species. As stated in the introduction, there are no reports of animal models exploring the efficacy of this agent in disease caused by this particular species. Clinical experience has anecdotally shown variable response rates of *C. krusei* to AFG. In the first open-label experience with AFG and candidemia, Krause et al. (7) reported five cases of disease caused by *C. krusei* infection, one of which was considered a microbiological failure at the end of therapy. The largest randomized trial of AFG for invasive candidiasis systematically excluded patients with infection by *C. krusei* since the comparator was FLU (18). Development of resistance while under treatment appears to be rare. Marr et al.

(5, 6) reported a *C. krusei* strain from a patient with leukemia that displayed reduced susceptibility to echinocandin drugs: the strain contained a heterozygous mutation of *FKS1*. Other echinocandins have consistently shown good activity against *C. krusei* in a limited number of patients in clinical trials (8, 10, 13, 15).

This study provides information regarding the in vivo activity of AFG against *C. krusei*. AFG seemed effective in controlling the infection in this murine model of disease, as evidenced by improved survival and reduced organism burden compared to placebo and other antifungals. Further clinical experience is warranted.

REFERENCES

1. **Clinical Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard CLSI document M27-A3. Clinical Laboratory Standards Institute, Wayne, PA.
2. **Garey, K. W., M. Rege, M. P. Pai, D. E. Mingo, K. J. Suda, R. S. Turpin, and D. T. Bearden.** 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin. Infect. Dis. **43:**25–31.
3. **Garey, K. W., R. S. Turpin, D. T. Bearden, M. P. Pai, and K. J. Suda.** 2007. Economic analysis of inadequate fluconazole therapy in non-neutropenic patients with candidaemia: a multi-institutional study. Int. J. Antimicrob. Agents **29:**557–562.
4. **Gumbo, T., G. L. Drusano, W. Liu, L. Ma, M. R. Deziel, M. F. Drusano, and A. Louie.** 2006. Anidulafungin pharmacokinetics and microbial response in neutropenic mice with disseminated candidiasis. Antimicrob. Agents Chemother. **50:**3695–3700.
5. **Hakki, M., J. F. Staab, and K. A. Marr.** 2006. Emergence of a *Candida krusei* isolate with reduced susceptibility to caspofungin during therapy. Antimicrob. Agents Chemother. **50:**2522–2524.
6. **Kahn, J. N., G. Garcia-Effron, M.-J. Hsu, S. Park, K. A. Marr, and D. S. Perlin.** 2007. Acquired echinocandin resistance in a *Candida krusei* isolate due to modification of glucan synthase. Antimicrob. Agents Chemother. **51:**1876–1878.
7. **Krause, D. S., J. Reinhardt, J. A. Vazquez, A. Reboli, B. P. Goldstein, M. Wible, and T. Henkel.** 2004. Phase 2, randomized, dose-ranging study evaluating the safety and efficacy of anidulafungin in invasive candidiasis and candidemia. Antimicrob. Agents Chemother. **48:**2021–2024.
8. **Kuse, E. R., P. Chetchotisakd, C. A. da Cunha, M. Ruhnke, C. Barrios, D. Raghunadharao, J. S. Sekhon, A. Freire, V. Ramasubramanian, I. Demeyer, M. Nucci, A. Leelarasamee, F. Jacobs, J. Decruyenaere, D. Pittet, A. J. Ullmann, L. Ostrosky-Zeichner, O. Lortholary, S. Koblinger, H. Diekmann-Berndt, and O. A. Cornely.** 2007. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. Lancet **369:**1519–1527.
9. **Marr, K. A.** 2004. Invasive Candida infections: the changing epidemiology. Oncology **18:**9–14.
10. **Mora-Duarte, J., R. Betts, C. Rotstein, A. L. Colombo, L. Thompson-Moya, J. Smietana, R. Lupinacci, C. Sable, N. Kartsonis, and J. Perfect.** 2002. Comparison of caspofungin and amphotericin B for invasive candidiasis. N. Engl. J. Med. **347:**2020–2029.
11. **Nucci, M., and K. A. Marr.** 2005. Emerging fungal diseases. Clin. Infect. Dis. **41:**521–526.
12. **Ostrosky-Zeichner, L., B. D. Alexander, D. H. Kett, J. Vazquez, P. G. Pappas, F. Saeki, P. A. Ketchum, J. Wingard, R. Schiff, H. Tamura, M. A. Finkelman, and J. H. Rex.** 2005. Multicenter clinical evaluation of the (1→3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. Clin. Infect. Dis. **41:**654–659.
13. **Ostrosky-Zeichner, L., D. Kontoyiannis, J. Raffalli, K. M. Mullane, J. Vazquez, E. J. Anaissie, J. Lipton, P. Jacobs, J. H. van Rensburg, J. H. Rex, W. Lau, D. Facklam, and D. N. Buell.** 2005. International, open-label, non-comparative, clinical trial of micafungin alone and in combination for treatment of newly diagnosed and refractory candidemia. Eur. J. Clin. Microbiol. Infect. Dis. **24:**654–661.
14. **Ostrosky-Zeichner, L., V. L. Paetznick, J. Rodriguez, E. Chen, and D. J. Sheehan.** 2007. Activity of anidulafungin (AFG) in a murine model of *Candida krusei* infection: evaluation of mortality and disease burden by quantitative tissue cultures and serum beta-glucan (BG) levels, abstr. M-1840, p. 457. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother.
15. **Pappas, P. G., C. M. Rotstein, R. F. Betts, M. Nucci, D. Talwar, J. J. De Waele, J. A. Vazquez, B. F. Dupont, D. L. Horn, L. Ostrosky-Zeichner, A. C. Reboli, B. Suh, R. Digumarti, C. Wu, L. L. Kovanda, L. J. Arnold, and D. N. Buell.** 2007. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. Clin. Infect. Dis. **45:**883–893.
16. **Perfect, J. R.** 2004. Antifungal resistance: the clinical front. Oncology **18:**15–22.
17. **Pfaller, M. A., D. J. Diekema, D. L. Gibbs, V. A. Newell, E. Nagy, S. Dobiasova, M. Rinaldi, R. Barton, A. Veselov, and the Global Antifungal Surveillance Group.** 2008. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J. Clin. Microbiol. **46:**515–521.
18. **Reboli, A. C., C. Rotstein, P. G. Pappas, S. W. Chapman, D. H. Kett, D. Kumar, R. Betts, M. Wible, B. P. Goldstein, J. Schranz, D. S. Krause, and T. J. Walsh.** 2007. Anidulafungin versus fluconazole for invasive candidiasis. N. Engl. J. Med. **356:**2472–2482.
19. **Vazquez, J. A., and J. D. Sobel.** 2006. Anidulafungin: a novel echinocandin. Clin. Infect. Dis. **43:**215–222.
20. **Wiederhold, N. P., L. K. Najvar, R. Bocanegra, D. Molina, M. Olivo, and J. R. Graybill.** 2007. In vivo efficacy of anidulafungin and caspofungin against *Candida glabrata* and association with in vitro potency in the presence of sera. Antimicrob. Agents Chemother. **51:**1616–1620.
21. **Zaas, A. K., and B. D. Alexander.** 2005. Echinocandins: role in antifungal therapy, 2005. Expert Opin. Pharmacother. **6:**1657–1668.