ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1996, p. 2907–2908 Vol. 40, No. 12
0066-4804/96/$04.00+0

Efficacy of Continuous Flucytosine Infusion against *Candida lusitaniae* in Experimental Hematogenous Murine Candidiasis

NICHOLAS C. KARYOTAKIS AND ELIAS J. ANAISSIE*

Department of Medical Specialties, Section of Infectious Diseases, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Received 18 June 1996/Returned for modification 29 July 1996/Accepted 9 September 1996

Candida lusitaniae **may cause life-threatening infections in the immunocompromised host and may be resistant to amphotericin B. Flucytosine (5-FC) is very active against *C. lusitaniae* isolates in vitro, while the in vivo response of murine infection to 5-FC is not as good. To evaluate the hypothesis that this discrepancy may be primarily due to the short half-life of 5-FC in mice, we compared the same total dosage of 75 mg of 5-FC per kg of body weight per day given by bolus injections or infused continuously via a subcutaneously implanted pump in immunosuppressed CF1 mice infected with *C. lusitaniae*. The fungal titers in the kidneys of mice treated with the continuous 5-FC infusion were significantly lower ($P \leq 0.05$) than those in the kidneys of mice that received bolus injections once or thrice daily. The antifungal activity of 5-FC against murine candidiasis is best evaluated when the drug is administered by continuous infusion.**

Fungal infections represent a significant cause of morbidity and mortality in immunocompromised patients (1, 8). Recently recognized opportunistic pathogens occasionally exhibit resistance to amphotericin B and the azoles (1, 4). *Candida lusitaniae* can cause life-threatening hematogenous infections in patients with hematologic malignancies. Management of these infections may be difficult because *C. lusitaniae* is frequently resistant to amphotericin B and exhibits variable susceptibility to other antifungal agents. In recent in vitro studies of 27 *C. lusitaniae* isolates, flucytosine (5-FC) was the most effective antifungal agent, with an MIC at which 90% of isolates are inhibited (MIC_{90}) of ≤0.125 mg/ml (5). Although a good correlation between the in vitro and in vivo antifungal activities of 5-FC has been demonstrated (3, 10), the effects of 5-FC on survival and clearance from the organs of mice infected with *C. lusitaniae* strains were not as impressive as would have been expected from the susceptibility results (3, 5). These suboptimal results may be due to the short half-life of 5-FC in mice, which leads to low concentrations of 5-FC in serum (7). In this study, we compared the efficacy of the same daily dose of 5-FC administered via continuous infusion with that of bolus injections administered once or thrice daily against hematogenous murine candidiasis caused by *C. lusitaniae.*

(This study was presented in part at Focus on Fungal Infections 5, San Francisco, Calif., March 1995.)

Osmotic pumps. Azlet osmotic pumps (Alza Corp., Palo Alto, Calif.) with a capacity of 2.5 ml delivering 10 μl/h (a continuous steady rate was achieved within 2 h after implantation) for a maximum of 10 days were used as the continuous subcutaneous infusion device.

Organism. A *C. lusitaniae* strain (CL 524) recovered from the blood of a patient cared for at The University of Texas M. D. Anderson Cancer Center, Houston, was used. The organism was maintained in water stock and was subcultured onto Sabouraud dextrose agar (SDA) plates. Inocula of *C. lusitaniae* for in vitro and in vivo studies were prepared by suspending the yeast cells in sterile 0.9% NaCl and adjusting the suspension to a 5% spectrophotometric transmission (Spectronic 20; Bausch & Lomb, Rochester, N.Y.). Colony counts of the inocula were verified by serial dilution on SDA plates. In vitro susceptibility testing of this organism was performed by a previously described broth microtiter method with agitation (2). The MIC of 5-FC for CL 524 was found to be 0.125 μg/ml.

Antifungal agent. For in vitro testing, 5-FC (Roche, Nutley, N.J.) in powder form was dissolved in dimethyl sulfoxide (Sigma, St. Louis, Mo.) and was diluted with RPMI 1640 (Sigma) to the highest concentration of 64 μg/ml. For animal experiments, 5-FC was dissolved in 5% glucose in water.

Animals. Four-week-old CF1 male mice (average weight, 25 g; Harlan-Sprague Breeding Laboratories, Indianapolis, Ind.) were used. The animal studies were performed in accord with the guidelines of the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* (9).

In vivo experiments. The mice were immunosuppressed with 5-fluorouracil (125 mg/kg of body weight given intraperitoneally) and cortisone acetate (125 mg/kg given subcutaneously) 24 h before the infectious challenge and received 75 mg of cortisone acetate per kg subcutaneously daily for the next 2 days. All animals were injected intravenously with the infecting inoculum containing 4×10^7 CFU of *C. lusitaniae* CL 524. Therapy began 1 h after inoculation and continued for 9 consecutive days. Infected mice were divided into groups of 10 each and were randomized to receive 75 mg of 5-FC per kg intraperitoneally once daily, 25 mg of 5-FC per kg intraperitoneally thrice daily, 75 mg of 5-FC per kg through a subcutaneously implanted pump, or no treatment. At 1 h before the inoculation, the osmotic pumps were surgically implanted in the subcutaneous space on the dorsum of each mouse by sterile technique and with the mouse under anesthesia with inhaled methoxyflurane.

Twenty-four hours after the end of treatment the mice were killed, and their kidneys were removed, weighed, and homogenized with 5 ml of sterile 0.9% NaCl in a stomacher blender (Seward Medical, London, United Kingdom). Samples from each specimen were serially diluted and plated onto SDA plates in duplicate. After 48 h of incubation at 35°C, the number of CFU per gram of tissue was calculated. The osmotic pumps were also retrieved from the dead animals and weighed; the weight of each pump was compared with that before im-

* Corresponding author. Mailing address: 6113 Charlotte St., Houston, TX 77005. Phone: (713) 661-4940. Fax: (713) 661-3813.

TABLE 1. Effect of continuous subcutaneous infusion of 5-FC compared with those of the same total dose given in one or three daily intraperitoneal bolus injections on fungal titers in the kidneys of transiently immunosuppressed CF1 mice with disseminated *C. lusitaniae* infection

Treatment group[a]	Log_{10} CFU/g (mean ± SE)
No treatment (control)	8.00 + 0.19
5-FC treatment	
75 mg/kg i.p. once daily	6.62 ± 0.21[b]
25 mg/kg i.p. three times daily	5.78 ± 0.16[b]
75 mg/kg s.c. daily via continuous infusion	5.17 ± 0.16[b,c]

[a] i.p., intraperitoneal; s.c., subcutaneous.
[b] $P \leq 0.05$ versus no treatment.
[c] $P \leq 0.05$ versus other treatment schedules.

plantation, and complete delivery of the drug was verified. The experiments were repeated once to confirm their reproducibility.

Statistical analysis. Data on fungal clearance from the organs of each group of mice were analyzed comparatively by the Mann-Whitney U test. Significance was defined as $P \leq 0.05$.

The fungal titers in the kidneys of all treated mice were significantly lower than those in the kidneys of untreated mice. Mice that received the continuous infusion of 5-FC had a significantly lower fungal burden in their kidneys than mice that received the same total dose of 5-FC given as bolus injections one or three times daily (Table 1). The fungal burden in the kidneys of mice treated with 5-FC injections three times daily was significantly lower than that in the kidneys of mice given the same total daily dose as one bolus injection. Hence, an incremental decrease in fungal titers in the kidneys was demonstrated, and this decrease depended on the frequency of 5-FC administration to the infected mice.

5-FC is one of the oldest available antifungal agents and has a broad spectrum of activity, but it is used infrequently today because of concern about myelosuppression, even though this effect rarely occurred in well-designed studies in which the level of the drug in the blood was monitored (6).

Our data confirm that 5-FC is effective against murine hematogenous *C. lusitaniae* infection. Our results demonstrate that in the treatment of hematogenous murine candidiasis, continuous infusion of 5-FC is more effective than an equal dose of 5-FC given as bolus injections once or thrice daily. The most likely explanation for this finding is the short elimination half-life (on the order of minutes) of 5-FC in mice (7). Hence, bolus injections of the drug result in short-lived peak concentrations followed by very low trough concentrations. A limitation of our study is the lack of actual data on 5-FC levels in the sera of treated mice. However, on the basis of the substantially enhanced efficacy of 5-FC in reducing the fungal burden in the kidneys of infected mice when it is administered continuously, it is reasonable to speculate that this mode of delivery results in improved pharmacokinetics.

We and other investigators have shown a good in vitro-in vivo correlation between the activities of antifungal agents against *Candida* spp., particularly *C. lusitaniae* (3, 5, 10). We found that 5-FC was the most active antifungal against *C. lusitaniae* in vitro. However, its in vivo efficacy against murine candidiasis was not as impressive as expected from the low MICs for the infecting isolates. In those in vivo studies, we administered 5-FC as bolus injections twice daily, and in view of our present results, we believe that this is the reason for the relatively modest benefit that the drug conferred to infected mice.

In conclusion, we believe that 5-FC is active against *C. lusitaniae* infections and that evaluation of its in vivo activity in murine models of infection in which the drug is given as bolus injections may underestimate the actual efficacy of this agent.

REFERENCES

1. **Anaissie, E.** 1992. Opportunistic mycoses in the immunocompromised host: experience at a cancer center and review. Clin. Infect. Dis. **14**(Suppl. 1)**:**S43–S53.
2. **Anaissie, E., V. Paetznick, and G. P. Bodey.** 1991. Fluconazole susceptibility testing of *Candida albicans*. Microtiter method that is independent of inoculum size, temperature, and time of reading. Antimicrob. Agents Chemother. **35:**1641–1646.
3. **Anaissie, E. J., N. C. Karyotakis, R. Hachem, M. C. Dignani, J. H. Rex, and V. Paetznick.** Correlation between in vitro and in vivo activity of antifungal agents against *Candida* species. J. Infect. Dis. **170:**384–389.
4. **Blinkhorn, R. J., D. Adelstein, and P. J. Spagnuolo.** 1989. Emergence of a new opportunistic pathogen, *Candida lusitaniae*. J. Clin. Microbiol. **27:**236–240.
5. **Dignani, M. C., N. C. Karyotakis, V. Paetznick, and E. J. Anaissie.** 1993. *Candida lusitaniae*: in vitro susceptibility and in vivo correlation in experimental murine candidiasis, abstr. A23. *In* Proceedings of the Conference on *Candida* and Candidiasis. American Society for Microbiology, Washington, D.C.
6. **Francis, P., and T. J. Walsh.** 1992. Evolving role of flucytosine in immunocompromised patients: new insights into safety, pharmacokinetics, and antifungal therapy. Clin. Infect. Dis. **15:**1003–1018.
7. **Graybill, J. (University of Texas Health Science Center).** Personal communication.
8. **Meunier-Carpentier, F., T. E. Kiehn, and D. Armstrong.** 1981. Fungemia in the immunocompromised host. Am. J. Med. **71:**363–370.
9. **National Institutes of Health.** 1985. Guide for the care and use of laboratory animals. National Institutes of Health, Bethesda, Md.
10. **Stiller, R. L., J. E. Bennett, H. J. Scholer, M. Wall, A. Polak, and D. A. Stevens.** 1983. Correlation of in vitro susceptibility test results with in vivo response: flucytosine therapy in a systemic candidiasis model. J. Infect. Dis. **147:**1070–1077.