ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1992, p. 1225–1229
0066-4804/92/061225-05$02.00/0

Vol. 36, No. 6

Combined Effect of Fluconazole and Recombinant Human Interleukin-1 on Systemic Candidiasis in Neutropenic Mice

BART-JAN KULLBERG, JAN W. VAN 'T WOUT, ROBBERT J. M. POELL, AND RALPH VAN FURTH*
Department of Infectious Diseases, University Hospital, Leiden, The Netherlands

Received 22 November 1991/Accepted 6 April 1992

The aim of the present study was to investigate the efficacy of treatment with a combination of fluconazole and human recombinant interleukin-1α (IL-1α) in normal or neutropenic mice with systemic *Candida albicans* infection. Six hours after intravenous injection of 5×10^4 CFU of *C. albicans* organisms, oral treatment twice daily with 2.5 or 10 mg of fluconazole per kg of body weight, a single intraperitoneal injection of 80 ng of IL-1, or a combination of the two was started. IL-1 had no influence on the antifungal activity of fluconazole in vitro or on the pharmacokinetics of fluconazole. For both normal and neutropenic mice, the number of *C. albicans* organisms cultured from the kidneys after 36 h of treatment was significantly lower in mice treated with IL-1 alone than in untreated animals. Treatment with fluconazole alone also significantly lowered the number of *C. albicans* organisms in the kidneys compared with that in untreated controls. In normal mice, the combination of fluconazole and IL-1 was not better than fluconazole alone. In neutropenic mice, combined treatment with IL-1 and 10 mg of fluconazole per kg led to significantly lower numbers of *C. albicans* organisms in the kidneys and the spleen than treatment with either agent alone. Although the precise mechanism by which IL-1 enhances resistance to infection is not clear, the additive effect of IL-1 and fluconazole in vivo indicates that combined therapy with immunomodulators and antifungal drugs is beneficial in immunocompromised mice with systemic fungal infections.

The treatment of systemic candidal infections in immunocompromised patients continues to be a major problem. In these patients, infections usually respond poorly to treatment with antifungal drugs, and cure is dependent on the reestablishment of host resistance (2, 5). These clinical observations are in agreement with experimental data indicating that neither amphotericin B nor the triazoles fluconazole and itraconazole are able to decrease the number of CFU in the kidneys of persistently neutropenic mice with systemic *Candida albicans* infection (20). These drugs were able to cure the infection in normal mice, indicating that intact host defense mechanisms are needed in order to obtain a fungicidal effect in vivo.

It is therefore rational to focus on the modulation of host defense mechanisms in addition to antifungal therapy in order to cure systemic candidiasis during neutropenia. We chose recombinant human interleukin-1α (IL-1α) as an immunomodulator, since we have shown that IL-1 is able to prolong survival of neutropenic mice with systemic *C. albicans* infection and inhibits the outgrowth of *C. albicans* in the kidneys, liver, and spleen of these mice even during severe, persistent neutropenia (6, 21). Fluconazole was chosen as an antifungal agent because this drug has proven to be at least as effective as amphotericin B in neutropenic animals (20) and is a relatively nontoxic drug with favorable pharmacokinetic properties. The aim of the present study was to assess the efficacy of the treatment of systemic *C. albicans* infections with the combination fluconazole and IL-1 and to investigate whether this combination has a fungicidal effect in vivo in neutropenic mice.

MATERIALS AND METHODS

Mice. Specific pathogen-free female Swiss Webster mice weighing 25 to 30 g (Broekman Institute, Someren, The Netherlands) were fed standard laboratory chow and water ad libitum.

C. albicans. *C. albicans* UC820, maintained on agar slants at 4°C, was inoculated into 100 ml of Sabouraud broth and cultured for 24 h at 37°C. After three washes with pyrogen-free saline by centrifugation at 1,500 × *g*, the number of yeasts was counted in a hemocytometer and the suspension was diluted to the appropriate concentration with pyrogen-free saline. The viability was confirmed by plating serial dilutions onto Sabouraud dextrose agar (SDA) plates.

Effect of fluconazole or IL-1 on the growth of *C. albicans* in vitro. Fluconazole (kindly donated by Pfizer Central Research, Sandwich, United Kingdom) was dissolved in distilled water as described previously (20). Recombinant human IL-1α, containing less than 20 pg of endotoxin per mg of protein, was a gift from Peter Lomedico (Hoffmann-La-Roche, Nutley, N.J.) *C. albicans* organisms (5×10^4/ml) were cultured in modified Eagle's medium (MEM, pH 7.3; GIBCO Ltd., Paisley, Scotland) supplemented with 10% newborn calf serum (GIBCO) in the presence of fluconazole (10 μg/ml), IL-1 (40 ng/ml), or a combination of fluconazole and IL-1. The suspension was incubated under 5% CO_2 at 37°C for 24 h. Growth curves were obtained by taking samples at various time points, plating 10-fold dilutions onto SDA, and counting the numbers of CFU after overnight incubation at 37°C. The MICs of fluconazole and IL-1, defined as the lowest concentrations that prevented visible growth after 24 h of incubation, were determined in MEM.

Infection model. Neutropenia was induced by means of two subcutaneous injections of cyclophosphamide (Astra Pharmaceutica BV, Rijswijk, The Netherlands): 150 mg/kg of body weight in 200 μl of phosphate-buffered saline (PBS) 4 days before injection of *C. albicans* and 100 mg/kg 1 day before injection of *C. albicans* (19). Each experiment included mice that had received no cyclophosphamide (henceforth called normal mice).

Mice received an intravenous injection of 5×10^4 CFU of

* Corresponding author.

C. albicans organisms in the lateral tail vein. Six hours after the injection of *C. albicans*, treatment with IL-1, fluconazole (at a dosage of either 2.5 or 10 mg/kg), or a combination of IL-1 and fluconazole was started. IL-1 was given as a single intraperitoneal (i.p.) injection of 80 ng of IL-1 with 2% newborn calf serum in 200 μl of pyrogen-free saline. Control animals received 200 μl of pyrogen-free saline with 2% newborn calf serum i.p. Fluconazole was administered orally via a feeding tube three times: 6, 18, and 30 h after the injection of *C. albicans*. Twelve hours after the last dose of fluconazole, the animals were killed by CO_2 asphyxia. Under sterile conditions, the left kidney, spleen, and liver were removed, weighed, and homogenized in sterile PBS in a Potter-Elvejhem glass tissue grinder. Serial 10-fold dilutions of these homogenates were prepared in PBS, and aliquots (0.1 ml) of appropriate dilutions were plated in duplicate onto SDA plates. After an overnight incubation at 37°C, the CFU were counted, and the result was expressed as the log CFU per gram of tissue.

Blood leukocyte counts. Blood samples (20 μl) taken from the retroorbital plexus were collected in plastic cups containing 40 μl of heparin (400 U/ml) (13). The leukocytes were counted in a Coulter Counter (model ZF; Coulter Electronics Ltd., Luton, England). The total numbers of granulocytes, lymphocytes, and monocytes per mm^3 were calculated from the total number of leukocytes per mm^3 and the differential counts of 200 leukocytes in two Giemsa-stained blood smears.

Pharmacokinetics. Neutropenic mice were given either a single dose of 80 ng of IL-1 or saline i.p. Either simultaneously with the injection of IL-1 or 24 h later, fluconazole (10 mg/kg) was administered orally. Blood was drawn by cardiac puncture 30 min and 1, 3, 5, 7, and 11 h later and collected in a heparinized tube. After centrifugation for 10 min at 1500 × *g*, the antifungal concentration in plasma was determined by an agar diffusion method using high-resolution antifungal assay medium (HR; Oxoid Ltd., Basingstoke, England) as described previously (20). *Candida pseudotropicalis* (Carlshalton strain) was used as the test organism. Standards were prepared in pooled murine plasma at concentrations ranging from 0.4 to 6.3 μg/ml.

Statistical analysis. The results obtained for the various treatment groups were analyzed by two-tailed Student's *t* tests and, when appropriate, by analysis of variance. For all comparisons, the level of significance between groups was set at $P < 0.05$.

RESULTS

Effect of fluconazole or IL-1 on the growth of *C. albicans* in vitro. When *C. albicans* was cultured in MEM, the number of CFU per ml, expressed as the geometric mean, increased from 5×10^4 to 2.68×10^6 in control medium, to 2.20×10^6 ($P > 0.05$) in the presence of IL-1 (40 ng/ml), and to 2.15×10^5 ($P < 0.001$ compared with control) in the presence of fluconazole (10 μg/ml) after 8 h of incubation. Fluconazole was not fungicidal even at concentrations of up to 100 μg/ml. The effect of the combination of IL-1 and fluconazole on the growth curve did not significantly differ from that of fluconazole alone. After 24 h of incubation, the same differences were seen (data not shown). Other concentrations of IL-1 (between 0.4 ng/ml and 4 μg/ml) also had no effect on the growth of *C. albicans*, nor did the repeated addition of IL-1 at different time points (data not shown). The same pattern was seen irrespective of whether fetal calf serum, normal mouse serum, or serum obtained from mice pretreated with 80 ng of IL-1 i.p. 24 h earlier was added to the culture medium (data not shown). The MICs against *C. albicans* were 0.8 μg/ml for fluconazole and >4.0 μg/ml for IL-1.

Effect of fluconazole or IL-1 in normal mice. In normal mice, the mean number of *C. albicans* organisms in the kidney was 3.58 ± 0.35 log CFU/g at the time that treatment began (Fig. 1a). Thirty-six hours later, the number of *C. albicans* organisms in the kidney had increased to 5.06 ± 0.40 log CFU/g in control mice, whereas the outgrowth in IL-1-treated animals was significantly lower (4.58 ± 0.44 log CFU/g; $P < 0.05$). At that time, the log CFU/g of kidney was 3.97 ± 0.44 after treatment with 2.5 mg of fluconazole per kg ($P < 0.005$) and 3.74 ± 0.37 after 10 mg of fluconazole per kg ($P < 0.001$ compared with control mice). The combination of IL-1 and fluconazole did not inhibit the outgrowth of *C. albicans* significantly more than did fluconazole alone (Fig. 1a).

In the liver and spleen of normal mice, there was no significant effect of fluconazole alone, irrespective of dose level, on the outgrowth of *C. albicans*, probably because the number of *C. albicans* organisms decreased spontaneously in control mice (Table 1). The combination of fluconazole and IL-1 was not more effective than fluconazole alone.

Effect of fluconazole or IL-1 in neutropenic mice. In neutropenic mice, the number of *C. albicans* organisms in the kidney 42 h after the injection was 6.37 ± 0.49 log CFU/g, which is significantly greater than in normal mice (Fig. 1b) ($P < 0.001$). In neutropenic mice treated with IL-1 alone, the number of *C. albicans* in the kidney (5.89 ± 0.43 log CFU/g) was lower than in neutropenic control mice ($P = 0.05$). The number of *C. albicans* organisms in the kidneys of mice treated with either dose of fluconazole was also significantly lower than in control mice (5.71 ± 0.42 log CFU/g; $P < 0.02$ for doses of 2.5 mg/kg and 5.52 ± 0.19 CFU/g; $P < 0.005$ for doses of 10 mg/kg). Combination therapy with IL-1 and 2.5 mg of fluconazole per kg was not significantly better than treatment with 2.5 mg of fluconazole per kg alone. After treatment with the combination of IL-1 and 10 mg of fluconazole per kg, the log CFU/g of kidney was 5.00 ± 0.37, which is significantly lower than after treatment with 10 mg of fluconazole per kg alone ($P < 0.005$) (Fig. 1b). Analysis of variance showed no significant interactions of treatment with IL-1 and treatment with fluconazole ($P > 0.8$), indicating that the observed effect is additive rather than synergistic.

The number of *C. albicans* organisms in the livers of neutropenic mice was significantly lower than in controls only for mice treated with 10 mg of fluconazole per kg (Table 1) ($P < 0.01$). The number of *C. albicans* organisms in the livers of mice treated with the combination of IL-1 and fluconazole was not lower than that found for mice that had received fluconazole alone.

In the spleen, neither IL-1 nor 2.5 mg of fluconazole per kg was able to reduce the number of *C. albicans* organisms compared with that of control mice (Table 1). The outgrowth of *C. albicans* was significantly reduced by treatment with 10 mg of fluconazole per kg ($P < 0.05$) or the combination of IL-1 and fluconazole (either 2.5 or 10 mg/kg; $P < 0.005$). For both doses of fluconazole, the combination with IL-1 was significantly more effective than fluconazole alone ($P < 0.05$). The results with mice injected with heat-inactivated (100°C for 20 min) IL-1 (data not shown) were similar to those with control mice injected with saline.

Blood leukocyte counts. Normal mice developed a marked granulocytosis 42 h after injection of *C. albicans* (Table 2). In mice treated with 10 mg of fluconazole per kg, the number of granulocytes at 42 h was significantly lower than in

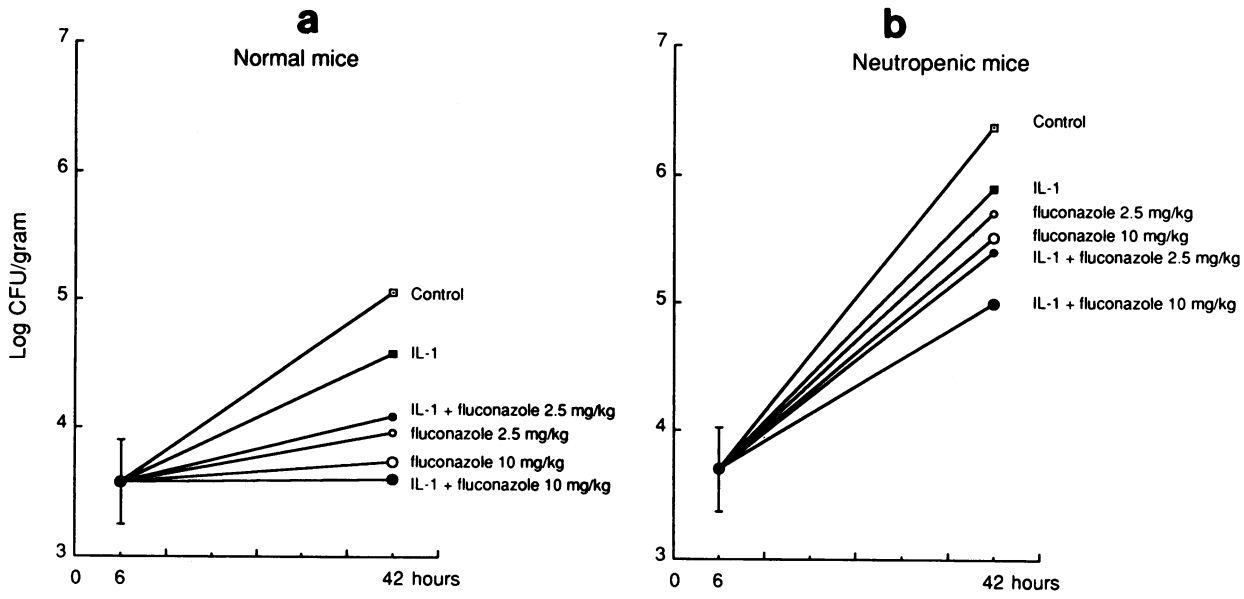

FIG. 1. Effect of fluconazole (○), IL-1 (■), or a combination of the two (●) on the number of *C. albicans* organisms in the kidneys of normal (a) and neutropenic (b) mice. Neutropenia was induced by two doses of cyclophosphamide on day −4 (150 mg/kg) and day −1 (100 mg/kg). Treatment was initiated 6 h after an intravenous injection of 5×10^4 CFU of *C. albicans* organisms. Fluconazole was given orally at 12-h intervals for 36 h, and IL-1 was given as a single i.p. injection of 80 ng 6 h after injection of the microorganisms. Each symbol represents the mean value for eight animals.

untreated controls ($P < 0.005$). The mean number of granulocytes in mice treated with IL-1 alone did not differ significantly from that in control mice, and the number in mice treated with the combination of IL-1 and fluconazole was not significantly different from that found for mice treated with fluconazole alone (Table 2). The effect of fluconazole on the number of granulocytes was dose dependent, since the number in mice treated with 2.5 mg of fluconazole per kg (data not shown) was intermediate to those for control mice and mice treated with 10 mg of fluconazole per kg.

The number of peripheral blood monocytes in normal mice had increased 42 h after injection of *C. albicans* in control mice and mice treated with IL-1 (Table 2). In mice treated with 10 mg of fluconazole per kg with or without IL-1, the number of monocytes after therapy did not differ significantly from that before treatment (Table 2).

The number of peripheral blood lymphocytes did not change significantly during the course of the infection, and no significant differences in the numbers of lymphocytes were found between the treatment groups (data not shown). In mice pretreated with cyclophosphamide, the numbers of granulocytes and monocytes remained very low throughout the experiment, and there were no significant differences between the treatment groups (Table 2).

Pharmacokinetics of fluconazole. Fluconazole was rapidly absorbed after oral administration. The calculated apparent elimination half-life was 3.4 h for control mice and 3.6 h for mice that received IL-1 i.p. simultaneously with oral flucon-

TABLE 1. Numbers of *C. albicans* organisms in spleens and livers of mice with disseminated *C. albicans* infection before and after treatment with IL-1, fluconazole, or both

Time of determination and therapy	No. of organisms (log CFU/g)[a] in:			
	Normal mice		Neutropenic mice[b]	
	Spleen	Liver	Spleen	Liver
Before treatment[c]	3.16 ± 0.39	3.39 ± 0.26	3.34 ± 0.20	3.32 ± 0.27
After treatment[d]				
None	2.97 ± 0.42	3.32 ± 0.28	4.30 ± 0.61	3.89 ± 0.47
IL-1	3.02 ± 0.25	3.20 ± 0.23	4.05 ± 0.44	3.61 ± 0.31
Fluconazole (2.5 mg/kg)	3.10 ± 0.21	3.17 ± 0.31	3.86 ± 0.13	3.84 ± 0.19
Fluconazole (10 mg/kg)	2.86 ± 0.30	3.06 ± 0.30	3.64 ± 0.24[e]	3.13 ± 0.52[e]
IL-1 + fluconazole (2.5 mg/kg)	2.80 ± 0.42	3.04 ± 0.30[e]	3.13 ± 0.49[e,f]	3.45 ± 0.55
IL-1 + fluconazole (10 mg/kg)	2.84 ± 0.16	3.09 ± 0.23	3.43 ± 0.24[e,f]	3.35 ± 0.25[e]

[a] Numbers represent the means (± standard deviations) for eight animals.
[b] 150 mg of cyclophosphamide per kg was injected subcutaneously 4 days before injecton of *C. albicans*, and 100 mg of cyclophosphamide per kg was injected subcutaneously 1 day before injection of *C. albicans*.
[c] Six hours after the injection of *C. albicans*.
[d] Forty-two hours after the injection of *C. albicans*.
[e] Value is significantly lower than that for mice that received no antifungal treatment ($P < 0.05$).
[f] Value is significantly lower than that for mice treated with the same dose of fluconazole without IL-1 ($P < 0.05$).

TABLE 2. Peripheral blood granulocyte and monocyte counts for mice with disseminated *C. albicans* infection before and after treatment with IL-1, fluconazole, or both

Time of determination and therapy	Counts (10^6/liter)[a] in:			
	Normal mice		Neutropenic mice[b]	
	Granulocytes	Monocytes	Granulocytes	Monocytes
Before infection	585 ± 205	104 ± 32	17 ± 12	19 ± 10
Before treatment of infection[c]	812 ± 217	55 ± 23	47 ± 15	24 ± 21
After treatment of infection[d]				
None	3,441 ± 265[e]	234 ± 63[e]	3 ± 3	14 ± 14
IL-1	2,806 ± 637[e]	295 ± 87[e]	24 ± 15	43 ± 31
Fluconazole (10 mg/kg)	1,501 ± 217[f]	52 ± 19	24 ± 17	0 ± 0
IL-1 + fluconazole (10 mg/kg)	1,441 ± 233[f]	85 ± 27	24 ± 19	28 ± 16

[a] Numbers represent the means (± standard errors of the mean) for four animals.
[b] Cyclophsphamide was subcutaneously injected 4 days before injection of *C. albicans* (150 mg/kg) and 1 day before injection of *C. albicans* (100 mg/kg).
[c] Six hours after the injection of *C. albicans*.
[d] Forty-two hours after the injection of *C. albicans*.
[e] Value significantly higher than value before treatment ($P < 0.05$).
[f] Value significantly lower than that for mice that received no antifungal treatment ($P < 0.05$).

azole (Fig. 2) ($P > 0.05$). The area under the curve (from 0 to 11 h) was 19.8 mg · h/liter for control mice and 17.5 mg · h/liter for IL-1-treated mice ($P > 0.05$). When the IL-1 was administered 24 h before the oral dose of fluconazole, the calculated half-life and the area under the curve were also similar to those obtained after administration of fluconazole alone (data not shown).

DISCUSSION

The main conclusion from the present study is that the combination of fluconazole and IL-1 has an additive effect against systemic candidiasis in neutropenic mice, although this combination was unable to cure the infection within 2 days.

It is known from earlier studies that the kidney is the target organ of systemic *C. albicans* infection in mice (9, 18). Eventually, normal mice are able to clear the yeasts from the liver and the spleen, but *C. albicans* continues to multiply in the kidneys, and renal insufficiency may ultimately lead to death (8, 9). In mice with cyclophosphamide-induced neutropenia, *C. albicans* continues to proliferate not only in the kidneys but also in the liver and spleen (reference 18 and present study). Treatment with fluconazole inhibits the outgrowth of *C. albicans* in neutropenic mice in a dose-dependent fashion, but this drug is not able to cause a decrease in the number of *C. albicans* organisms compared with that at the start of treatment. The same limitations in neutropenic mice have been described for itraconazole and amphotericin B (20). We have no explanation for the fact that fluconazole and IL-1 have an additive effect in the kidneys and the spleen but not in the liver of neutropenic mice.

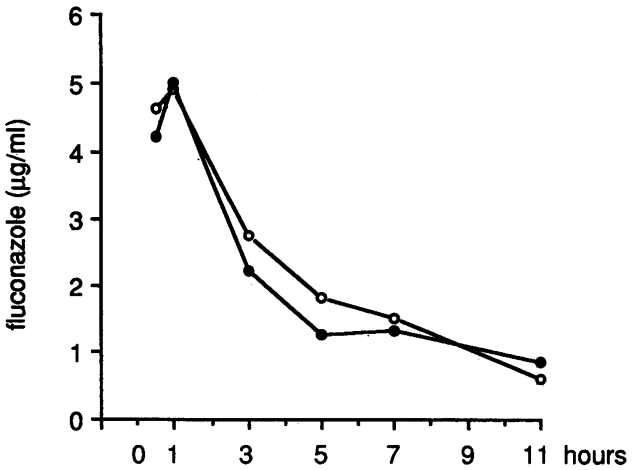

FIG. 2. Concentration in plasma of fluconazole after oral administration of 10 mg/kg to neutropenic mice that simultaneously received either 80 ng of IL-1 (●) or saline (○) i.p. Each symbol represents the mean for three mice.

Nakamura et al. reported a synergistic effect of combination treatment with IL-1 and amphotericin B on the survival of normal mice with a systemic *C. albicans* infection (10). Their study is difficult to interpret because amphotericin B was administered orally, which means that effective concentrations at the site of infection probably were not achieved. Moreover, the outcome was measured in terms of survival and not confirmed by assessment of the outgrowth of *C. albicans* in the organs (10). The present study may provide more insight into the use of a combination of immunomodulators and antimicrobial drugs, which is a new approach to the treatment of infectious diseases. The timing of the administration of both the antifungal agent and the immunomodulator in experimental infection models has to be chosen carefully. In the case of IL-1, it is known from earlier studies that a single dose has an optimal effect on the course of infections when given 24 h before the injection of the microorganisms (6, 17, 21). However, since the inhibitory effect of IL-1 on the outgrowth of *C. albicans* is already apparent during the first day of the infection, mice pretreated with IL-1 would have lower numbers of *C. albicans* organisms than control mice at the moment that antifungal therapy is begun. This difference would hinder comparison of the subsequent course of the infection in the two treatment groups. Therefore, in the present study the first doses of IL-1 and fluconazole were given at the same time, 6 h after the injection of *C. albicans*. On the other hand, the interval between the injection of *C. albicans* and treatment was long enough for the infection to become established in the various organs. The fact that IL-1 has proven to be effective when given up to 6 h after the onset of infection (6, 21) enabled us to study the effect of fluconazole and IL-1 as therapeutic rather than prophylactic agents.

The mechanism by which IL-1 enhances resistance to infections is not clear as yet (6, 17). A direct anticandidal effect of IL-1 in vitro was ruled out earlier (21). In the present study, we were not able to demonstrate an effect of IL-1 on the fungistatic activity of fluconazole in vitro or on the pharmacokinetics of fluconazole. Although IL-1 plays a role in the regulation of the production and release of granulocytes (1, 4, 15), we demonstrated earlier that IL-1-mediated resistance to *C. albicans* infections is not dependent on the presence of granulocytes (6, 7). In the present study, IL-1 did not significantly influence the number of peripheral blood granulocytes either, and the additive effect of the combination of fluconazole and IL-1 was observed in persistently neutropenic mice. Normal mice developed a granulocytosis during the *C. albicans* infection. In mice treated with fluconazole, the granulocytosis was reduced, probably as a result of the resolution of the infection due to fluconazole.

The administration of IL-1 to patients entails several problems. First, IL-1 is an important mediator of fever and the acute-phase response (3, 11), and administration to humans causes various adverse reactions such as fever, chills, myalgia, and hypotension (14, 16). Therefore, the treatment of patients with the combination of IL-1 and an antifungal drug cannot yet be advocated. Second, IL-1 is not effective when given 24 h after the onset of the *Candida* infection in experimental models (6, 12, 17). In patients, the infection is usually well established before it has been diagnosed, and thus therapy with an immunomodulator such as IL-1 may be too late. Other immunomodulators that are effective when the infection is already established should be investigated in combination with antifungal drugs for treatment of fungal infections.

ACKNOWLEDGMENTS

We thank Herman Mattie for helpful suggestions.

This study was supported financially by Pfizer Central Research, Sandwich, United Kingdom.

REFERENCES

1. **Annema, A., W. Sluiter, and R. Van Furth.** 1992. Effect of interleukin-1, macrophage colony-stimulating factor, and factor increasing monocytopoiesis on the production of leukocytes in mice. Exp. Hematol. **20**:69–74.
2. **Bodey, G. P.** 1984. Candidiasis in cancer patients. Am. J. Med. **77**:13–19.
3. **Dinarello, C. A., J. G. Cannon, J. W. Mier, H. A. Bernheim, and G. LoPreste.** 1986. Multiple biological activities of human recombinant interleukin-1. J. Clin. Invest. **77**:1734–1739.
4. **Fibbe, W. E., J. Van Damme, A. Billiau, H. M. Goselink, P. J. Voogt, G. Van Eeden, P. Ralph, B. Altrock, and J. H. F. Falkenburg.** 1988. Interleukin 1 induces human marrow stromal cells in long-term culture to produce granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor. Blood **71**:430–435.
5. **Horn, R., B. Wong, T. E. Kiehn, and D. Armstrong.** 1985. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. Rev. Infect. Dis. **7**:646–655.
6. **Kullberg, B. J., J. W. Van 't Wout, and R. Van Furth.** 1990. Role of granulocytes in enhanced host resistance to *Candida albicans* induced by recombinant interleukin-1. Infect. Immun. **58**:3319–3324.
7. **Kullberg, B. J., J. W. Van 't Wout, and R. Van Furth.** 1991. No effect of recombinant human interleukin-1 on the numbers of peripheral blood and peritoneal leukocytes during an acute inflammation. Inflammation **15**:457–470.
8. **Leunk, R. D., and R. M. Moon.** 1979. Physiological and metabolic alterations accompanying systemic candidiasis in mice. Infect. Immun. **26**:1035–1041.
9. **Louria, D. B.** 1985. Candida infections in experimental animals, p. 29–51. *In* G. P. Bodey and V. Fainstein (ed.), Candidiasis. Raven Press, New York.
10. **Nakamura, S., A. Minami, K. Fujimoto, and T. Kojima.** 1989. Combination effect of recombinant human interleukin-1α with antimicrobial agents. Antimicrob. Agents Chemother. **33**:1804–1810.
11. **Okusawa, S., J. A. Gelfand, T. Ikejima, R. J. Connolly, and C. A. Dinarello.** 1988. Interleukin-1 induces a shock-like state in rabbits. J. Clin. Invest. **81**:1162–1172.
12. **Pecyk, R. A., E. B. Fraser-Smith, and T. R. Matthews.** 1989. Efficacy of interleukin-1β against systemic *Candida albicans* infections in normal and immunosuppressed mice. Infect. Immun. **57**:3257–3258.
13. **Sluiter, W., E. Hulsing-Hesselink, I. Elzenga-Claasen, and R. Van Furth.** 1985. Method to select mice in the steady state for biological studies. J. Immunol. Methods **760**:135–143.
14. **Smith, J., W. Urba, R. Steis, J. Janik, B. Fenton, W. Sharfman, K. Conlon, M. Sznol, S. Creekmore, N. Wells, L. Elwood, J. Keller, K. Hestdal, C. Ewel, J. Rossio, W. Kopp, M. Shimuzu, J. Oppenheim, and D. Longo.** 1990. Interleukin-1 alpha (IL-1α): results of a phase I toxicity and immunomodulatory trial. Proc. Am. Soc. Clin. Oncol. Annu. Meet. **9**:717.
15. **Stork, L. C., V. M. Peterson, C. H. Rundus, and W. A. Robinson.** 1988. Interleukin-1 enhances murine granulopoiesis in vivo. Exp. Hematol. **16**:163–167.
16. **Tewari, A., W. C. Buhles, Jr., and H. F. Starnes, Jr.** 1990. Preliminary report: effects of interleukin-1 on platelet counts. Lancet **336**:712–714.
17. **Van der Meer, J. W. M., M. Barza, S. M. Wolff, and C. A. Dinarello.** 1988. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal Gram-negative infection. Proc. Natl. Acad. Sci. USA **85**:1620–1623.
18. **Van 't Wout, J. W., I. Linde, P. C. J. Leijh, and R. Van Furth.** 1988. Contribution of granulocytes and monocytes to resistance against experimental disseminated Candida albicans infections. Eur. J. Clin. Microbiol. Infect. Dis. **7**:736–741.
19. **Van 't Wout, J. W., I. Linde, P. C. J. Leijh, and R. Van Furth.** 1989. Effect of irradiation, cyclophosphamide, and etoposide (VP-16) on number of peripheral blood and peritoneal leukocytes in mice under normal conditions and during acute inflammatory reaction. Inflammation **13**:1–14.
20. **Van 't Wout, J. W., H. Mattie, and R. Van Furth.** 1989. Comparison of the efficacies of amphotericin B, fluconazole, and itraconazole against systemic *Candida albicans* infection in normal and neutropenic mice. Antimicrob. Agents Chemother. **33**:147–151.
21. **Van 't Wout, J. W., J. W. M. Van der Meer, M. Barza, and C. A. Dinarello.** 1988. Protection of neutropenic mice from lethal Candida albicans infection by recombinant interleukin 1. Eur. J. Immunol. **18**:1143–1146.