ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1996, p. 2221–2223 Vol. 40, No. 9
0066-4804/96/$04.00+0

Effects of Cyclophosphamide and Ceftriaxone on Gastrointestinal Colonization of Mice by *Candida albicans*

G. SAMONIS,[1]* N. C. KARYOTAKIS,[2] E. J. ANAISSIE,[3] E. BARBOUNAKIS,[1] S. MARAKI,[1] Y. TSELENTIS,[1] AND G. P. BODEY[3]

Division of Medicine, The University of Crete, Heraklion 71110, Greece,[1] Department of Internal Medicine, The University of Texas Medical School,[2] and Department of Medical Specialties, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030[3]

Received 22 January 1996/Returned for modification 15 March 1996/Accepted 1 July 1996

Adult male Crl:CD1(ICR) mice were fed chow containing *Candida albicans* to induce sustained gastrointestinal colonization by the yeast. Groups of mice were rendered neutropenic with cyclophosphamide and subsequently received ceftriaxone, while other groups received normal saline and served as controls. Stool cultures were obtained immediately before and at the end of treatment. The administration of cyclophosphamide substantially increased the *C. albicans* counts in the stools of mice. The addition of ceftriaxone to the cyclophosphamide regimen did not significantly increase the level of gastrointestinal colonization by *C. albicans*. There was no evidence of *Candida* dissemination to internal organs.

Infectious complications, and fungal infections in particular, remain a major risk for cancer patients (1). Among these infections disseminated candidiasis is very prominent, and *Candida albicans* remains the major pathogen (1, 3). The source of disseminated candidiasis is primarily the gastrointestinal tract of the patient, which is frequently colonized by yeasts (1, 3). Broad-spectrum antimicrobial agents customarily used for the treatment of febrile neutropenia in patients with cancer substantially alter the bacterial flora of the gut and, thus, facilitate the uninhibited growth of yeasts (1, 3, 14). Among these agents, ceftriaxone is excreted in the bile, resulting in high intestinal concentrations, and can significantly increase the alimentary tract colonization by yeasts in experimental animals and humans (11). On the other hand, antineoplastic agents causing neutropenia and mucositis, such as cyclophosphamide, damage the natural host defenses and predispose the patients to disseminated fungal infection (1).

We have described an adult mouse model of sustained colonization of the gastrointestinal tract with *C. albicans* (12). We successfully used this model to mimic the conditions in cancer patients and predict the level of the gastrointestinal yeast colonization after the administration of various agents (13, 14). In the present study we have evaluated the effects of cyclophosphamide alone, as well as in combination with ceftriaxone, on the colonization of the gastrointestinal tract of mice by *C. albicans.*

One hundred thirty Crl:CD1(ICR) BR male mice, 3 months old and weighing approximately 30 g each (Charles River Laboratories, Wilmington, Mass.), were used. Animal experimentation was performed in accordance with the standards of the Animal Welfare Act and the guidelines of the National Institutes of Health.

Seventy mice were fed special chow containing *C. albicans* for 15 days, as described previously (12). Sustained gastrointestinal colonization by the yeast was confirmed 7 days after the end of the special diet by quantitative stool cultures (12). The remaining 60 mice were fed regular chow which did not contain *C. albicans*, and the yeast could not be recovered from their stools. Subsequently, 50 mice of the *Candida* colonized group were given a dose of 150 mg cyclophosphamide (Bristol-Myers, Evansville, Ind.) per kg of body weight every 84 h to a total of 4 doses. Peripheral blood from 2 randomly selected cyclophosphamide-treated mice was collected from the retroorbital capillary plexus every third day after the initiation of the treatment, and leukocyte and neutrophil counts were performed in a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.). Sustained neutropenia (<500 polymorphonuclear cells per ml) was achieved 3 days after the first dose of cyclophosphamide and was maintained for a minimum of 10 days. Twenty of the cyclophosphamide-treated mice were also given ceftriaxone (Roche, Nutley, N.J.) at a dose of 11 mg per mouse, which is equivalent to a dose of 2 g in humans (6). Ceftriaxone was administered subcutaneously every 24 h starting 3 days after the first cyclophosphamide dose and continuing for 10 consecutive days. Both cyclophosphamide and ceftriaxone were purchased commercially and were handled according to their respective manufacturer's recommendations. The remaining 20 *Candida*-colonized mice were divided in two control groups of 10 animals each. The first group received 0.9% NaCl in the same schedule, volume, and route as mice given cyclophosphamide alone, while the second group received 0.9% NaCl in the same manner as the cyclophosphamide- and ceftriaxone-treated animals.

The 60 noncolonized mice were divided in 4 groups that also served as controls. Twenty mice received cyclophosphamide, and 20 received cyclophosphamide and ceftriaxone in the same dose-schedule as the colonized animals. Of the remaining 20 noncolonized mice, 10 received 0.9% NaCl in the same schedule, volume, and route as the cyclophosphamide-treated mice, and 10 received 0.9% NaCl in the same schedule, route, and volume as the cyclophosphamide- and ceftriaxone-treated animals.

Cultures from the stools of mice were obtained before the initiation of treatment and on the last day of antibiotic administration. Five randomly selected mice from each experimental group were sacrificed within 24 h of the last ceftriaxone dose. The animals were dissected and their heart, lungs, liver, kidneys, and spleen were removed and processed as previously

* Corresponding author. Mailing address: Department of Medical Oncology, Division of Medicine, The University of Crete, P.O. Box 1393, Heraklion 71110, Greece. Phone: (81) 269-747. Fax: (81) 269-802.

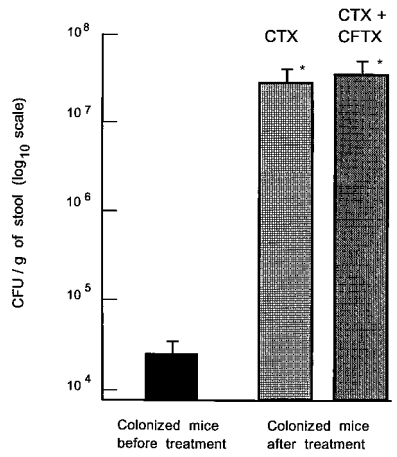

FIG. 1. Effects of 10 days of treatment with cyclophosphamide (CTX) alone or in combination with ceftriaxone (CFTX) on the level of gastrointestinal colonization of mice by *C. albicans*. Results represent means ± standard errors. *, $P < 0.05$ versus colonized mice before treatment.

described (11) in order to detect *C. albicans* in these tissues by mycological culture and histopathology.

Results from quantitative stool cultures were compared by using an analysis of variance at a level of significance of 0.05.

The median concentration of *C. albicans* in the stools of mice fed chow containing the yeast before the initiation of cyclophosphamide treatment was $2.58 \times 10^4 \pm 0.58 \times 10^4$ CFU/g of stool. The administration of cyclophosphamide and the combination of cyclophosphamide with ceftriaxone significantly increased the level of colonization of the alimentary tract of mice by *C. albicans* to the range of 2×10^7 CFU/g of stool (Fig. 1). The coadministration of cyclophosphamide and ceftriaxone resulted in an increase of the gastrointestinal colonization of mice which was comparable to that obtained by cyclophosphamide alone ($P > 0.05$) and to our previous findings with ceftriaxone alone (11). As expected the administration of normal saline did not affect the concentration of the yeast in the stools of the colonized mice. *C. albicans* was not detected in the stools of the noncolonized animals treated with cyclophosphamide, with or without ceftriaxone, or normal saline (data not shown).

Results from organ and tissue cultures were negative except for one *C. albicans*-colonized and cyclophosphamide-treated mouse whose lungs grew 100 CFU of yeast per g of tissue and another colonized and cyclophosphamide- and ceftriaxone-treated mouse whose lungs and spleen yielded 1,500 CFU and 150 CFU of *C. albicans* per g of tissue, respectively. Histopathological examination of these organs failed to reveal tissue invasion by the yeast. Thus, we could not demonstrate that the documented increase in the level of gastrointestinal colonization of mice by *C. albicans* led to invasive candidiasis under the experimental conditions used. Histopathological examination of the gastrointestinal tract of mice treated with cyclophosphamide did not reveal significant mucositis. Whether higher cyclophosphamide doses and more prolonged immunosuppression would have induced higher yeast titers in the gastrointestinal tract and possible dissemination is unknown. Previous studies have documented translocation of *Candida* spp. only from the alimentary tract of colonized infant mice (5, 10) or from mice subjected to irradiation and corticosteroids (4, 15), cytarabine doses causing gastrointestinal mucosal damage (16), or burn injuries (8), suggesting that immaturity or disruption of the intestinal mucosal barrier plays a pivotal role in the dissemination of the yeasts. Our results indicate that cyclophosphamide plays an important and independent role in increasing the level of gastrointestinal colonization by *C. albicans*. The observed increase was significant and comparable to that produced by broad-spectrum antibacterial agents (11, 13).

To the best of our knowledge these are the first data suggesting that a commonly used antineoplastic agent may affect the colonization of the gastrointestinal tract by *Candida* spp. irrespective of the coadministration of antibiotics. The general immunosuppressive effects of cyclophosphamide on murine candidiasis are well documented and consist of depression of the numbers and candidacidal activity of peripheral blood polymorphonuclear cells, monocytes, and lymphocytes, as well as diminished ability to form antibodies (2, 9). However, the effects of cyclophosphamide on local immunologic parameters of the gastrointestinal tract remain largely unknown. In view of our results it would be reasonable to speculate a depression of local immunologic mechanisms (i.e., intestinal immunoglobulins and cytotoxic cells) by cyclophosphamide that allowed the rapid proliferation of intraluminal yeasts. An alternative explanation would be a possible direct antimicrobial effect of cyclophosphamide against the normal gastrointestinal flora. However, since cyclophosphamide and its active metabolites are converted to inactive compounds by hepatic and intestinal mucosal enzymes, a direct effect on gastrointestinal microorganism seems unlikely. In addition, an in vitro study of chemotherapeutic agents against gram-negative bacteria (including common gut-colonizing organisms) showed that cyclophosphamide had minimal activity (7). Further studies are clearly needed in order to validate our results in humans and to clarify the specific host-*Candida* spp. interactions in the gastrointestinal tract, which is the major reservoir of yeasts and the nidus of disseminated infections in cancer patients.

REFERENCES

1. **Anaissie, E.** 1992. Opportunistic mycoses in the immunocompromised host: experience at a cancer center and review. Clin. Infect. Dis. **14**(Suppl. 1)**:**S43–53.
2. **Bistoni, F., M. Baccarini, E. Blasi, P. Marconi, P. Puccetti, and E. Garaci.** 1983. Correlation between in vivo and in vitro studies of modulation of resistance to experimental *Candida albicans* infection by cyclophosphamide in mice. Infect. Immun. **40:**46–55.
3. **Bodey, G. P.** 1984. Candidiasis in cancer patients. Am. J. Med. **77** (Suppl. 4D)**:**13–19.
4. **Cole, G. T., K. T. Lynn, and K. R. Seshan.** 1990. Evaluation of a murine model of hepatic candidiasis. J. Clin. Microbiol. **28:**1828–1841.
5. **Field, L. H., L. M. Pope, G. T. Cole, M. N. Guentzel, and L. J. Berry.** 1981. Persistence and spread of *Candida albicans* after intragastric inoculation of infant mice. Infect. Immun. **31:**783–791.
6. **Freireich, E., E. A. Gehan, D. P. Rall, L. H. Schmidt, and H. E. Skipper.** 1966. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother. Rep. **50:**219–244.
7. **Goldschmidt, M. C., and G. P. Bodey.** 1972. Effect of chemotherapeutic agents upon microorganisms isolated from cancer patients. Antimicrob. Agents Chemother. **1:**348–353.
8. **Inoue, S., J. A. Wirman, J. W. Alexander et al.** 1988. *Candida albicans* translocation across the gut mucosa following burn injury. J. Surg. Res. **44:**479–492.
9. **Moser, S. A., and J. E. Domer.** 1980. Effects of cyclophosphamide on murine candidiasis. Infect. Immun. **27:**376–386.
10. **Pope, L. M., G. T. Cole, M. N. Guentzel, and L. J. Berry.** 1979. Systemic and gastrointestinal candidiasis of infant mice after gastrointestinal challenge. Infect. Immun. **25:**702–707.
11. **Samonis, G., E. J. Anaissie, and G. P. Bodey.** 1990. Effects of broad-spectrum antimicrobial agents on yeast colonization of the gastrointestinal tracts of mice. Antimicrob. Agents Chemother. **34:**2420–2422.
12. **Samonis, G., E. J. Anaissie, B. Rosenbaum, and G. P. Bodey.** 1990. A model of sustained gastrointestinal colonization by *Candida albicans* in healthy adult mice. Infect. Immun. **58:**1514–1517.
13. **Samonis, G., H. Anastassiadou, M. Dassiou, Y. Tselentis, and G. P. Bodey.** 1994. Effects of broad-spectrum antibiotics on colonization of the gastroin-

testinal tracts of mice by *Candida albicans*. Antimicrob. Agents Chemother. **38:**602–603.

14. **Samonis, G., A. Gikas, P. Toloudis, S. Maraki, G. Vrentzos, Y. Tselentis, N. Tsaparas, and G. P. Bodey.** 1994. Prospective study of the impact of broad spectrum antibiotics on the yeast flora of the human gut. Eur. J. Clin. Microbiol. Infect. Dis. **13:**665–667.
15. **Umenai, T., S. Konno, and N. Ishida.** 1979. Systemic candidiasis from *Candida albicans* colonizing the gastrointestinal tract of mice. Experientia **35:** 1331–1332.
16. **Wingard, J. R., J. D. Dick, W. G. Merz, G. R. Sandford, R. Saral, and W. H. Burns.** 1980. Pathogenicity of *Candida tropicalis* and *Candida albicans* after gastrointestinal inoculation in mice. Infect. Immun. **29:**808–813.