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Effects of Broad-Spectrum Antimicrobial Agents on Yeast Colonization of the Gastrointestinal Tracts of Mice

GEORGE SAMONIS, ELIAS J. ANAISSIE, AND GERALD P. BODEY*

Department of Medical Specialties, Section of Infectious Diseases, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

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Male Crl:CD1 (ICR) BR mice, 3 months old, were fed regular chow or chow containing *Candida albicans*. Subsequently, both groups were treated with either antibiotics or normal saline for 10 days. Stool cultures were obtained to determine the extent of *C. albicans* colonization immediately before treatment, at the end of treatment, and 1 week after the discontinuation of treatment. Animals in the antibiotic-treated groups had substantially higher *Candida* counts than control animals fed *C. albicans*, especially if they received ceftriaxone, cefoperazone, or ticarcillin-clavulanic acid. There was no evidence of *Candida* dissemination to internal organs.

Cancer patients are at high risk of developing disseminated candidiasis (2, 18, 19, 28). In many patients, the gastrointestinal tract is the source of dissemination (2, 13, 29). Treatment with broad-spectrum antibiotics increases the risk of candidiasis in susceptible hosts by increasing the concentration of *Candida* organisms in the gastrointestinal tract. We recently described an adult mouse model of sustained gastrointestinal colonization by *Candida albicans* (27). In the present study, we report the effects of seven commonly used antibacterial agents on the level of gastrointestinal colonization by *C. albicans* in this adult mouse model.

Fifty Crl:CD1 (ICR) BR mice, 3 months old and weighing approximately 30 g each (Charles River Laboratories, Wilmington, Mass.), were used in experiments with each antibiotic. The gastrointestinal tracts of 30 of these mice were colonized with *C. albicans* by feeding the mice for 2 weeks with chow containing the yeast by a method described previously (27). The remaining 20 mice of each group were fed regular chow not containing *C. albicans*. The level of colonization was verified 1 week after the feeding period by stool cultures (27). Subsequently, 20 mice of each group colonized with *C. albicans* were injected subcutaneously with the study antibiotic for 10 days. The remaining 10 colonized mice were injected (subcutaneously) with the same volume (30 μl) of saline for the same duration. Also, 10 mice each of the noncolonized group were injected for 10 days with either the same antibiotic or 30 μl of saline. The latter three groups served as controls.

The antibiotics used were ceftriaxone, cefoperazone, ceftazidime, ticarcillin-clavulanic acid, imipenem-cilastatin, gentamicin, and aztreonam and were supplied by their commercial manufacturers. The dosage schedules were equivalent to those of humans and were calculated by the method of Freireich et al. (7). The equivalence of antibiotic dosage schedules for humans and mice is shown in Table 1. Stool cultures were performed on day 10 of treatment for all animals and 1 week after the end of treatment for surviving mice.

Five randomly selected mice of each group were sacrificed by cervical dislocation and dissected on day 10 of treatment. The lungs, heart, liver, kidneys, and spleen were separately weighed and homogenized in 10 ml of saline by using a Stomacher Lab Blender 80 (Tekmar Co., Cincinnati, Ohio). A 100-μl amount of the suspension was then spread onto plates containing tryptic soy agar with 5% sheep blood (Regional Media Laboratories, Lenexa, Kans.) and Sabouraud dextrose agar with cycloheximide and chloramphenicol (BBL Microbiology Systems, Cockeysville, Md.). The plates were incubated at 37°C for 48 h. Histopathologic examination was performed on the lungs, liver, spleen, kidneys, adrenal glands, stomach, intestines, and mesenteric lymph nodes, with special emphasis on the detection of invasion by *C. albicans*.

The median concentration of *C. albicans* prior to antibiotic treatment in the stools of the mice fed *C. albicans* was $10^{4.2}$ CFU/g (range, $10^{3.9}$ to $10^{4.3}$ CFU/g). After antibiotic administration, the level of gastrointestinal colonization of mice by *C. albicans* varied depending on the antibiotic used. The medians and ranges of *C. albicans* concentrations in the stools of the *C. albicans*-colonized, antibiotic-treated mice on day 10 of treatment are presented in Table 2. Ceftriaxone, cefoperazone, and ticarcillin-clavulanic acid had a major impact on the level of colonization, with increases in the concentrations of *C. albicans* of 10^3 to 10^4 CFU. On the other hand, imipenem-cilastatin, gentamicin, and ceftazidime increased the colonization by only 10^2 CFU and aztreonam increased the concentration by only 10^1 CFU. In addition, ceftriaxone and ticarcillin-clavulanic acid caused more sustained increases of the concentrations of *C. albicans* in the gastrointestinal tract 1 week after discontinuation of antibiotic treatment. The concentrations of *C. albicans* in the colonized mice which were injected with saline remained essentially unchanged. No *C. albicans* was found in the stools of the noncolonized mice injected with either antibiotic or saline. There was no histopathologic or microbiologic evidence of *Candida* infection in any of the examined organs. *Candida* organisms were found only in the intestinal luminal contents of the mice fed *C. albicans* but without evidence of tissue invasion.

Our data indicate that there was a substantial difference in the levels of gastrointestinal tract colonization by *C. albicans* depending on the antibiotic used. Drugs with high intestinal concentrations and broad-spectrum activities (ceftriaxone, cefoperazone, ticarcillin-clavulanic acid) (3, 6, 11, 14, 22) were associated with the highest increases, as opposed to drugs with low intestinal concentrations or more

* Corresponding author.

TABLE 1. Equivalence of antibiotic dosage schedule(s) in humans and mice

Antibiotic	Daily dosage schedule	
	70-kg human	30-g mouse
Cetriaxone	2 g q24h[a]	11 mg q24h
Cefoperazone	4 g q8h	21 mg q8h
Ceftazidime	2 g q8h	10 mg q8h
Ticarcillin-clavulanic acid	3.1 g q4h	12 mg q4h
Imipenem-cilastatin	1 g q6h	4.5 mg q8h
Gentamicin	120 mg q8h	0.6 mg q8h
Aztreonam	2 g q6h	10.5 mg q6h

[a] q24h, Every 24 h.

limited antibacterial activities (gentamicin, ceftazidime, aztreonam) (5, 10, 15, 21, 28, 30). Imipenem, which has the broadest spectrum of activity but does not reach significant intestinal concentrations, did not cause a high increase in *C. albicans* colonization (20, 24, 25). Findings similar to ours have been reported for both animals and humans (1, 4, 8, 9, 12, 16, 17, 23, 26, 31). In humans, cefoperazone (8, 17), ceftriaxone (9), and broad-spectrum penicillins such as piperacillin (8) and ampicillin plus clavulanic acid (31) have caused substantial increases in gastrointestinal colonization by yeasts, while ceftazidime was associated with a modest increase (16, 26). This occurs without the administration of *C. albicans*, suggesting that humans normally are colonized by this yeast at low concentrations. The use of gentamicin in mice was not associated with any increase (12). The similarities between our findings with this mouse model and the above-mentioned reports suggest that this model could be useful in predicting the degree of gastrointestinal colonization in humans after antibacterial therapy.

On the basis of the pharmacokinetics and tissue distributions of antibacterial agents and their effects on the gut flora, it appears that two phenomena would have to be realized for a substantial increase in the yeast concentration to occur. First, the drug has to get into the gastrointestinal tissue and contents, and second, it has to have a broad spectrum of antibacterial activity.

In conclusion, in this mouse model, yeast colonization increases dramatically after exposure to agents with high gastrointestinal tract concentrations and broad-spectrum antibacterial activities. These findings may improve our understanding of the pathogenesis of invasive candidal infections in febrile neutropenic patients receiving broad-spectrum antibiotics.

TABLE 2. Effects of antibiotics on gastrointestinal colonization of mice by *C. albicans*[a]

Antibiotic	Median *C. albicans* concn (range) (CFU/g of stool)	
	Day 10 of treatment	1 wk after end of treatment
Ceftriaxone	$10^{7.3}$ ($10^{6.9}$–$10^{7.6}$)	$10^{6.4}$ ($10^{5.9}$–$10^{6.5}$)
Cefoperazone	$10^{8.2}$ ($10^{7.9}$–$10^{8.3}$)	10^{5} ($10^{4.7}$–$10^{5.5}$)
Ceftazidime	$10^{6.9}$ ($10^{6.8}$–$10^{7.2}$)	$10^{4.8}$ ($10^{4.8}$–$10^{5.1}$)
Ticarcillin-clavulanic acid	$10^{8.7}$ ($10^{7.9}$–$10^{8.6}$)	$10^{7.1}$ ($10^{6.8}$–$10^{7.3}$)
Imipenem-cilastatin	$10^{6.3}$ ($10^{6.0}$–$10^{6.5}$)	$10^{4.9}$ ($10^{4.7}$–$10^{5.2}$)
Gentamicin	$10^{6.2}$ ($10^{6.2}$–$10^{6.5}$)	$10^{4.6}$ ($10^{4.2}$–$10^{4.6}$)
Aztreonam	$10^{5.3}$ ($10^{5.1}$–$10^{5.5}$)	$10^{3.8}$ ($10^{3.8}$–$10^{4.2}$)

[a] CFU/g of stool before treatment: $10^{4.2}$ (median) and $10^{3.9}$ to $10^{4.3}$ (range).

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