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Suppression by *Saccharomyces boulardii* of Toxigenic *Clostridium difficile* Overgrowth after Vancomycin Treatment in Hamsters

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***Saccharomyces boulardii* prevented the development of high counts of *Clostridium difficile*, high titers of toxin B, and positive latex agglutination tests after cessation of vancomycin treatment for hamsters. The protocol used was designed to simulate relapse of human *C. difficile*-associated colitis. *S. boulardii* was protective in this model.**

Vancomycin is commonly used to treat *Clostridium difficile*-induced pseudomembranous colitis (PMC), and while therapy is usually successful, relapse of PMC is common (10, 16, 17, 23), with rates of 11 to 50% previously reported (5, 9, 22, 25). Browne et al. (6) and Bartlett et al. (4, 5) have shown that daily administration of vancomycin to hamsters protects them from clindamycin-induced death; however, once treatment is stopped, they succumb to cecitis caused by overgrowth of toxigenic *C. difficile*. We modified this procedure to obtain a reproducible animal model of the relapse of PMC that occurs in humans after cessation of vancomycin treatment. This model was used to study the effect of *Saccharomyces boulardii*, a nonpathogenic yeast which had previously been shown to decrease *C. difficile*-associated cecitis and death (13, 19). We hypothesized that it could decrease the rate of PMC relapse after cessation of vancomycin treatment.

Hamsters were given a single, potentially lethal oral dose of clindamycin (10 mg/kg), after which they were protected from cecitis by a vancomycin solution (0.35 mg/ml) as the source of drinking water for 10 days. Of the 18 animals given only water, 13 succumbed to cecitis within 6 days of the dose of clindamycin; there were no deaths among the vancomycin-treated animals (data not shown). It was found that the development of high counts ($>10^6$) of *C. difficile* after vancomycin treatment was variable. Therefore, a toxigenic strain of *C. difficile* from a moribund, unprotected hamster was isolated, and a washed-cell suspension ($\sim 10^3$ CFU) was used to orally inoculate all animals every 48 h after stopping vancomycin treatment. At the end of the 10-day vancomycin treatment, some animals were given a 5% suspension (5×10^8 CFU/ml) of *S. boulardii* (made fresh daily) as a source of drinking water (19), while the others received only water.

At designated times (Table 1), groups of animals were sacrificed, and the cecal contents were analyzed for *C. difficile*, toxin B, and latex agglutination test (LAT) positivity. Three experiments were performed. *C. difficile* counts were determined by serial dilution of hamster cecal material in prereduced supplemental peptone broth (Becton Dickinson Vacutainer Systems, Rutherford, N.J.) and anaerobic growth of diluted samples on Difficile agar plates (Prepared Media Laboratories, Tualatin, Oreg.). The characteristic *C. difficile* colonies were counted after 48 h of incubation. Representative colonies were confirmed as *C. difficile* by the gas chromatographic procedure of Allen et al. (1). LATs were done by using the Culturette rapid latex agglutination assay kit (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.). The results were ranked from 0 to 4, depending upon the amount of agglutination (20). Toxin B titers were determined as described by Willey and Bartlett (24), except that human embryonic tonsil cells were used as the tissue culture cell layer. Significant differences between treatment groups were assessed by using Student's *t* test for *C. difficile* counts and toxin B and the Wilcoxon ranked sum test for the protein detected by the LAT.

With this model, animals reproducibly became infected with *C. difficile* ($>10^5$ CFU) after vancomycin treatment. Significant differences between *C. difficile* counts for controls and treated animals were observed for those time points at which the average log counts were greater than seven for controls. The highest average *C. difficile* log count for yeast-treated animals was 6.6. The most striking effect of yeast treatment was the decrease in toxin B and in the number of animals that were LAT positive (Table 1). Of the yeast-treated animals tested in all experiments, only 2 of 66 (3%) were positive in the LAT, and 2 of 73 (3%) were positive for toxin B. In contrast, 22 of 66 (33%) control animals were positive in the LAT, and 37 of 73 (51%) were positive for toxin B. The original purpose of the LAT was to detect toxin A (enterotoxin); however, the specificity of the LAT has been questioned (12). Recent evidence has shown that the LAT reacts with an as-yet-uncharacterized protein of low molecular weight and not with the enterotoxin. This protein is associated with *C. difficile* and toxin B and may be of some use for detection purposes (3, 14).

Further work is needed to determine whether the sharp reduction in toxin titers is a result of the lower *C. difficile* counts in yeast-treated hamsters or a direct action of *S. boulardii* on the toxins. While these experiments were not designed to measure mortality as an endpoint, some hamsters died from diarrhea and cecitis before the time of sacrifice (Table 1). After vancomycin treatment was stopped, 18 of 120 animals receiving water died, along with 1 of 117 animals receiving the yeast.

Our efforts have focused on modalities to prevent the antibiotic-mediated decline of resistance of the gut ecosystem ("colonization resistance" [21]) to overgrowth by *C. difficile*. We propose that *S. boulardii* has potential as an exogenously administered agent for maintaining colonization resistance during antibiotic therapy because this yeast (i) is not affected by antibacterial antibiotics or sulfonamides, (ii) is nonpathogenic, (iii) has demonstrated antagonistic activity (in vitro and in vivo) against various bacterial pathogens (7) and *Candida* spp. (7, 8, 15), and (iv) has been shown to

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TABLE 1. *C. difficile*, toxin B, and LAT results for treated and control hamsters

Days after stopping vancomycin	Expt	Treatment	*C. difficile*		Toxin B		No. LAT positive/total
			No. positive[a]/total	Count[b]	No. positive/total	Titer[c]	
0	3		0/7				
5	3	Water	1/10	5.3	0/10	0	0/10
	3	Yeast	3/10	5.9	0/10	0	0/10
7	3	Water	10/10	7.4[d]	4/10	1.0	5/10
	3	Yeast	9/10	5.8	1/10	3.0	1/10
9	3	Water	10/10	7.6[d]	1/10	3.0	4/10[d]
	3	Yeast	10/10	6.6	1/10	1.0	0/10
	2	Water	10/10	7.0[d]	9/10	3.9[d]	2/10
	2	Yeast	9/10	5.4	0/10	0	0/10
	1	Water	9/10	5.1	10/10	2.6[d]	1/3
	1	Yeast	9/10	6.0	0/10	0	1/3
12	3	Water	3/3	5.8	3/3	1.0	1/3
	3	Yeast	3/3	5.4	0/3	0	0/3
	2	Water	9/10	6.1	0/10	0	2/10
	2	Yeast	10/10	5.5	0/10	0	0/10
	1	Water	10/10	8.8[d]	10/10	2.6[d]	7/10[d]
	1	Yeast	9/10	5.7	0/10	0	0/10
Total (%)	All	Water	85		51		33
		Yeast	85		3		3

[a] Number of animals with $>10^3$ CFU/g of cecal contents.
[b] Average (log CFU per gram) of positive samples.
[c] Average (log of reciprocal of highest dilution that exhibited cytotoxicity) of positive samples.
[d] $P < 0.05$.

decrease antibiotic-associated diarrhea in clinical trials in humans (2, 11, 18). Previous work (13, 19) has demonstrated a decrease in mortality for *S. boulardii*-treated hamsters given clindamycin (an animal model for human PMC). The present study employed a protocol designed to simulate the relapse frequently observed after vancomycin treatment of human PMC. The results confirmed our hypothesis that *S. boulardii* administration would help prevent extensive overgrowth of *C. difficile*. An unexpected finding was the near absence of *C. difficile* toxins in treated animals. Intestinal colonization resistance enhanced by *S. boulardii* should be tested as a modality for diminishing the high incidence of PMC relapse after vancomycin treatment for humans.

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