Influence of Antibiotics on the Recovery and Kinetics of *Saccharomyces boulardii* in Rats

Alan V. Boddy,[1,2] Gary W. Elmer,[3,4] Lynne V. McFarland,[3] and René H. Levy[1]

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Saccharomyces boulardii (SB) is a yeast that is used for the prevention and treatment of antibiotic-associated diarrhea and for the treatment of pseudomembranous colitis. Since SB will most commonly be used when the bacterial flora of the gastrointestinal tract have been disrupted by antibiotic treatment, the influence of different antibiotics on the kinetics and recovery of SB in feces was investigated in rats. Following a single oral dose, SB concentrations in feces were measured for periods of 1 to 6 days. Although SB is eliminated exclusively in the feces, less than 3% of the dose is recovered as viable yeast. When rats were treated with neomycin, which is active against gram-negative aerobic bacteria but not against anaerobes, no change was observed in recovery of SB when compared with recovery from untreated rats. Also, there was no change in the rate at which SB concentrations declined in feces. In contrast, treatment with clindamycin and the broad-spectrum antibiotic ampicillin, which are active against anaerobes, produced an increase in the recovery of SB of up to seven times that of controls and slowed the rate of decline of SB concentration in the feces. This antibiotic effect on SB disposition was also found when SB was administered in multiple doses. An eightfold increase in the steady-state output of SB was observed from ampicillin-treated animals. Analysis of the recovery and kinetic data showed that the primary effect of these antibiotics was to reduce the destruction of SB, probably in the cecum and colon. These studies indicate that viable cells of SB are likely to be present at the highest concentration under those conditions where its action is needed, i.e., in the antibiotic-treated gastrointestinal tract.

KEY WORDS: *Saccharomyces boulardii;* antibiotics; gastrointestinal kinetics; interaction; fecal recovery.

INTRODUCTION

Saccharomyces boulardii (SB) is a yeast used in the treatment and prevention of diarrhea associated with antibiotic use (1–3). Activity has also been demonstrated in the treatment of pseudomembranous colitis (4). This potentially fatal disease is due to overgrowth of toxigenic strains of *Clostridium difficile* (5,6). While the mechanism of action of SB is as yet undetermined, the protective effect in animal models of pseudomembranous colitis is associated with a minimum effective concentration of living SB in the lumen of the intestine (7–9).

An initial study of the kinetics of SB in rats and humans showed that less than 1% of an oral dose is recovered in feces and that SB disappeared rapidly from the gastrointestinal tract on cessation of dosing (10). In gnotobiotic mice, however, SB appeared to colonize the gastrointestinal tract after a single dose and was detectable in the feces at a constant level for 60 days. Administration of a dilution of human feces to these animals resulted in a rapid decline in SB in feces (11). Thus, the microbial content of the gastrointestinal tract appears to have a significant effect on SB survival. Since patients requiring treatment with SB will already possess a disturbed intestinal microflora due to antibiotic treatment, it appeared necessary to determine how such treatment affects the kinetics and recovery of the yeast. The aim of the present study was to investigate this interaction in rats and to provide some insight into the mechanisms of the processes affecting SB during its passage through the gastrointestinal tract.

MATERIALS AND METHODS

SB was obtained from Laboratoires Biocodex, Montrouge, France. It was supplied as a lyophilized powder which is reconstituted in distilled water for use. Neomycin and ampicillin were obtained from Sigma, and clindamycin was a gift from the Upjohn Company, Kalamazoo, MI.

Male Sprague–Dawley rats were used, weighing 180 to 220 g at the start of the study. Rats were kept in individual metabolic cages for the duration of the experiments. They were fed a yeast-free regimen and water *ad libitum* and were kept on an approximately constant 12-hr light/dark cycle, allowing for sampling.

The experimental design included two studies. In the first, the effect of treatment with the antibiotics neomycin, ampicillin, and clindamycin on the recovery and kinetics of a single dose of SB in feces was investigated. The second study focused on the effect of ampicillin on the steady-state kinetics of SB. In the single-dose studies, animals were studied in groups of eight in a crossover experimental protocol. Half of each group received antibiotic treatment in the first phase, the other half in the second phase. There was a washout period of 1 week between phases.

In treated animals, antibiotics were administered in drinking water, at a concentration of 0.5 g/liter, for 2 days before administration of SB and throughout the period of fecal collection. A suspension of SB in distilled water (0.3–0.5 ml) was administered by oral intubation to provide a dose of 4×10^9 colony-forming units per kilogram of body weight (CFU/kg). Total fecal output was collected at 4 to 12 hr intervals after the dose of SB. Except for samples collected during the evening, fecal samples were analyzed within 1 hr of collection. Evening samples were held at 5°C until analysis the following day. The feces were weighed and diluted 1:10 in water and assayed for SB. The total viable SB count (CFU) of each sample was determined as described previously (12), except that Sabouraud dextrose agar (Difco) containing 1% tartaric acid was used as the selective medium. From the SB count (CFU) and the weight of feces, the yeast

[1] Department of Pharmaceutics, University of Washington, BG-20, Seattle, Washington 98195.

[2] Present address: Pharmacogenetics Research Unit, Department of Pharmacological Sciences, University of Newcastle Upon Tyne, Newcastle Upon Tyne, U.K. NE2 4HH.

[3] Department of Medicinal Chemistry, University of Washington, BG-20, Seattle, Washington 98195.

[4] To whom correspondence should be addressed.

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output and the yeast concentration (CFU/g) for each sample was calculated. The cumulative recovery of SB, as a percentage of the administered dose, was calculated from the sum of CFU measurements in all the fecal samples. The average rate of output of feces was also calculated.

In the neomycin and ampicillin experiments, a dose of a nonabsorbed marker, Brilliant Blue adsorbed onto methylcellulose as described by Lutwak and Burton (13), was also administered with SB. Fecal samples were diluted 1:100 or 1:1000 in water and the absorbance (AU) measured at 630 nm.

First-order rate constants were determined for the rate of decline of marker in the feces and for the rate of decline of SB, following an initial pulse of SB in the first two or three fecal samples. This terminal rate constant was used to calculate a half-life for the disappearance of SB from the gastrointestinal tract.

The SB multiple-dose study was conducted to determine the effect of ampicillin on the steady-state fecal levels of SB. In treated animals, ampicillin was given as described above for 2 days before the administration of SB and throughout the experimental period. SB was given daily (4×10^9 CFU/kg) for a total of 8 days. Stool samples were collected as described above from day 3 to day 8. Daily SB output was compared between the two groups.

Theory

Various models of gastrointestinal transit have been described (14,15), but usually these models have been applied to fecal data in ruminants and so may not be appropriate for other species. An empirical model of SB kinetics in the GI tract has been introduced previously (10) and is developed further here to establish the relationship between SB concentration in feces and events in the GI tract (SB binding, flow of GI contents, and destruction of SB).

The rate of appearance of material in the feces is equal to the product of the rate of flow of feces and the concentration of the material in the GI tract that is available to be transported by fecal flow. Thus,

$$\frac{A_{i(\mathrm{F})}}{T_i} = \frac{G_{i(\mathrm{F})}}{\mathrm{T_i}} \times Cu[T_{i(\mathrm{mid})} - T_\mathrm{l}] \tag{1}$$

where $A_{i(\mathrm{F})}$ is the amount of SB collected in $G_{i(F)}$ grams of feces in the i^{th} collection interval of duration T_i, and Cu is the concentration of SB available to be transported at some time T_l prior to the midpoint of the collection interval, $T_{i(\mathrm{mid})}$. Rearranging Eq. (1) gives

$$\frac{A_{i(\mathrm{F})}}{G_{i(\mathrm{F})}} = Cu[T_{\mathrm{i(mid)}} - T_\mathrm{l}] \tag{2}$$

Thus, the concentration of SB in feces (CFU/g) during a sampling period is equal to the available concentration of SB in the GI tract at some previous time $[T_{\mathrm{i(mid)}} - T_\mathrm{l}]$. The time lag, T_l, is assumed to be small relative to the collection interval and will be assumed to be constant for all the collection intervals.

The rate of decline of SB in the GI tract is assumed to be a first-order process due to the sum of removal by fecal flow and to destruction of the yeast. Thus, the rate of decline of Cu at time t after administration may be described by

$$\frac{dCu(t)}{dT} = f_\mathrm{u}\frac{dC(t)}{dT} = -f_\mathrm{u} \times Cu(t) \times (K_\mathrm{LF} + K_\mathrm{LD}) \tag{3}$$

where C is the total concentration of SB in the GI tract and f_u is the fraction of C that is unbound and available for movement or destruction. The rate constant K_LF describes the physical removal of unbound SB from the colon by flow of intestinal contents and K_LD is the rate constant describing destruction of unbound SB (10). This model assumes that the processes are linear and that the relevant part of the GI tract behaves approximately as a well-stirred compartment prior to formation of feces.

Integrating Eq. (3) and substituting for $CU(t)$ and t from Eq. (2) gives

$$\frac{A_{i(\mathrm{F})}}{G_{i(\mathrm{F})}} = C_\mathrm{l}e^{-K_\mathrm{a}[T_{i(\mathrm{mid})} - \mathrm{T_l}]} \tag{4}$$

where C_I represents some initial concentration of SB in the relevant section of the GI tract and K_a is the apparent rate constant for the decline of SB in feces $[K_\mathrm{a} = f_\mathrm{u}(K_\mathrm{LD} + K_\mathrm{LF})]$. From Eq. (4) it can be shown that the total recovery of SB in feces, F, is a function of the rate constants for flow and for destruction of SB:

$$F = \frac{K_\mathrm{LF}}{K_\mathrm{LF} + K_\mathrm{LD}} \tag{5}$$

Equations (5) and (4) establish the two parameters (recovery and terminal rate constant, respectively) used in Table I to evaluate the effects of neomycin, ampicillin, and clindamycin. The relative importance of K_LF and K_LD is considered in the Discussion.

RESULTS

Figures 1–3 show the time course of SB concentration in feces for the single-dose studies. Generally, the yeast could be detected in feces for 3 to 6 days, depending on the antibiotic treatment received. No SB could be detected in feces during the first 12 hr following an oral dose (preliminary experiments, data not shown). Thereafter, SB concentrations rose rapidly to about 10^7 CFU/g (controls and neomycin-treated animals) or 10^8 to 10^9 CFU/g (ampicillin- and clindamycin-treated animals) at about 12 to 20 hr after the dose (Figs. 1–3). The decline of SB in the feces following this pulse was approximately log-linear. None of the complex models which have been proposed to describe the kinetics of gastrointestinal transit (12,13) provided an adequate fit to the entire SB data set.

The total recovery of SB in feces in control animals and in the same animals treated with antibiotics is shown in Table I. No significant difference was detected between the two phases of the crossover in the recovery or rate of decline of SB (data not shown). The lack of a phase effect indicated that the washout period was sufficient for the gut flora to return to normal, with respect to metabolism of the yeast, after antibiotic treatment, and that the administration of SB

Table I. Effect of Antibiotics on the Recovery and Kinetics of *S. boulardii* in Rat Feces After a Single Oral Dose[a]

	Treatment						
	Neomycin	Control	Ampicillin	Control	Clindamycin	Control	Marker
Fecal output rate (g/hr)	0.463 (0.118)	0.378 (0.067)	0.481 (0.087)	0.355 (0.054)	0.532 (0.348)	0.450 (0.072)	
	NS		$P < 0.05$		NS		
Recovery, F(% dose)	1.97 (2.07)	0.82 (0.84)	14.1 (6.0)	2.95 (2.26)	7.74 (2.39)	0.84 (0.95)	
	NS		$P < 0.001$		$P < 0.001$		
Terminal rate constant, K_a (hr)$^{-1}$	0.433 (0.067)	0.492 (0.126)	0.096 (0.016)	0.279 (0.072)	0.097 (0.045)	0.251 (0.030)	0.051 (0.028)
	NS		$P < 0.01$		$P < 0.01$		
Half-life (hr)	1.60	1.41	7.22	2.48	7.14	2.76	13.6

[a] Recovery and rate constants given as mean (with standard deviation for comparison). Fecal output rates averaged over entire collection interval. Half-lives calculated from mean value of K_a. P values refer to significance level for difference between control and treated animals using nonparametric Mann–Whitney U test. NS, not significant.

had no lasting effect on the flora. Therefore, data from the two crossover phases were combined ($n = 8$) for the purpose of calculating the values in Table I and Figs. 1–3.

The control data indicated that in the absence of antibiotics, only a small percentage of SB survives the passage through the gut (Table I). These recoveries are consistent with previous investigations with SB in rat and man (10,16). The mean recovery associated with neomycin treatment was higher than control, but this difference was not statistically significant. Both ampicillin and clindamycin treatments were associated with significant increases in the recovery of SB. Expressed as a percentage of control, recovery was increased by 500 to 700%.

The rate of decline of SB in feces was relatively consistent for the control animals (Table I). It was not possible to obtain a good estimate for this parameter in every case due to the rapid decline of SB in feces of control and neomycin-treated rats. As with the recovery data, neomycin treatment had no significant effect on the rate of decline of SB in feces (Table I and Fig. 1). Ampicillin and clindamycin treatment produced a significant decrease in the rate of decline of SB, extending the time during which the yeast could be detected in the feces by several days (Figs. 2 and 3). The value of the apparent rate constant for decline of SB in animals treated with ampicillin and clindamycin approached that obtained with the nonabsorbed marker (Table I). A log(absorbance)–time profile for the marker in feces is shown in Figs. 1 and 2.

The effect of ampicillin on SB recovery found in the single-dose experiment was also observed in rats administered SB daily for 8 days. Steady-state SB output was achieved after about 3 days of daily SB dosing (data not shown) and remained until the experiment was terminated at

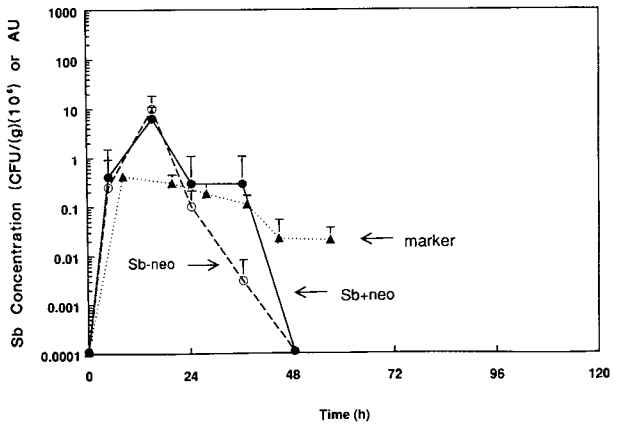

Fig. 1. Semilog plot of SB concentration in feces and absorbance of marker in fecal dilutions (AU) against midtime of collection interval. The data are from neomycin-treated or control animals combined for the two phases of a crossover study. Solid line, neomycin-treated; dashed line, controls; dotted line, absorbance of the marker. $N = 8$ for each curve.

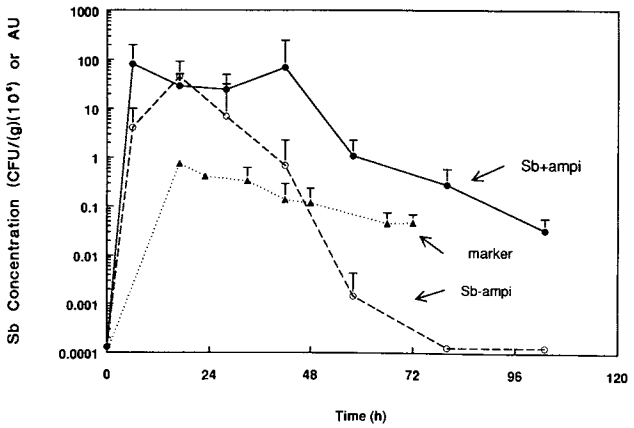

Fig. 2. Semilog plot of SB concentration in feces and absorbance of marker in fecal dilutions (AU) against midtime of collection interval. The data are from ampicillin-treated or control animals combined for the two phases of a crossover study. Solid line, ampicillin treated; dashed line, controls; dotted line, absorbance of the marker. $N = 8$ for each curve.

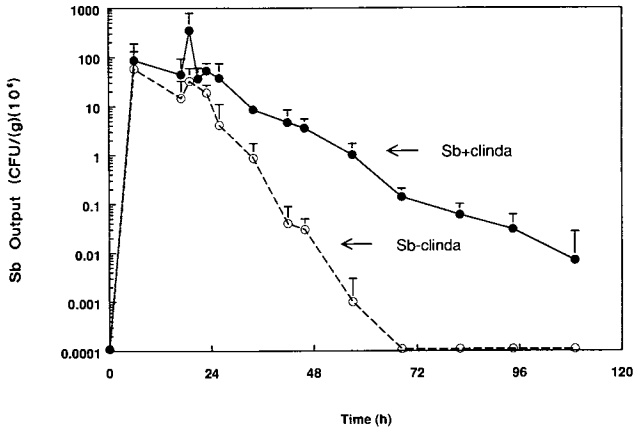

Fig. 3. Semilog plot of SB concentration in feces against midtime of collection interval. The data are from clindamycin-treated or control animals for the two phases of a cross over study. Solid line, clindamycin-treated; dashed line, controls. $N = 8$ for each curve.

day 8 (Fig. 4). Ampicillin-treated animals had substantially higher steady-state fecal concentrations than their controls, i.e., 164 ± 14 vs 21 ± 19 (CFU/12 hr) (10^6).

DISCUSSION

Despite the successful clinical use of SB in the treatment of antibiotic-associated gastrointestinal disorders, little is known about the kinetics of the yeast in the gastrointestinal tract in the presence of antibiotics. Also, the mechanism of destruction of SB has not yet been elucidated. It has been stated that SB is not sensitive to the acid pH of the stomach (17), although another study demonstrated in rats that the amount of SB leaving the stomach was only 10% of the dose administered (16). In the latter study, this fraction remained intact until it reached the cecum, where a further decrease in SB counts was observed. Since the administration of antibiotics is unlikely to have had an effect on gastric pH or on gastric emptying rate, it appears that the principle site of interaction between antibiotics and SB would be in the cecum or colon. Thus, Eq. (4) may be taken to describe

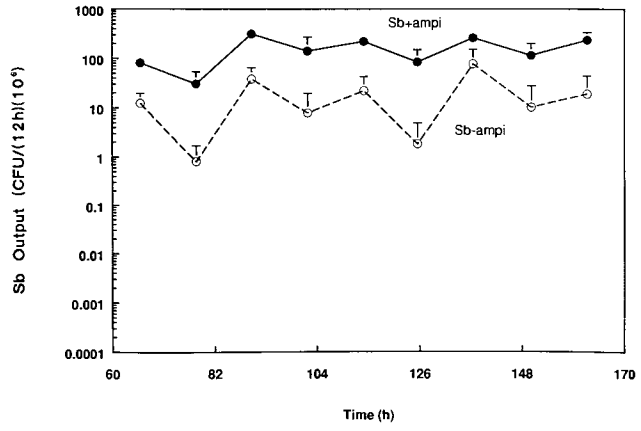

Fig. 4. Steady-state fecal output of SB. Semilog plot of SB output at 12-hr intervals. Animals were dosed every 24 hr starting at time = 0. Solid line, ampicillin-treated; dashed line, controls.

the destruction of SB in the cecum or colon and its removal into the feces, where it can be measured.

The rate of decline of SB in the feces reflects the rate of decline in the gastrointestinal tract [Eq. (2)]. Therefore, the differential effects of these antibiotics give some insight into the kinetics and mechanism of destruction of SB in the gastrointestinal tract. Since the recovery of SB in feces is so low, it can be inferred from Eq. (5) that the predominant process governing the disappearance of SB from the gastrointestinal tract is destruction ($K_{LD} \gg K_{LF}$). In the single-dose experiments, treatment with ampicillin and clindamycin increased the recovery and slowed the rate of decline of SB in feces, apparently by reducing K_{LD}. The half-life of SB in the gastrointestinal tract was increased from about 2.5 hr in control animals to about 7.1 hr in animals treated with ampicillin or clindamycin. This may be compared with a half-life of 13.6 hr for the marker. If destruction of the yeast were inhibited completely ($K_{LD} = 0$), SB concentrations should decline in parallel with the marker (provided that the marker adequately represents the movement of SB in the gastrointestinal tract). In contrast, total recovery of SB in feces is limited by the destruction of SB in the stomach. The rate of fecal output (g/hr) was increased up to 40% by antibiotic treatment, but this was not sufficient to account for the sevenfold increase in recovery. Neomycin, however, had no effect on recovery or on the rate of decline of SB. The multiple-dose study showed that the increase in recovery associated with ampicillin treatment persists and is reproducible for several consecutive days.

The different antimicrobial activities of the antibiotics studied provide some evidence for the mechanism of destruction of SB in the gastrointestinal tract. Neomycin is not active against anaerobic microorganisms. Most anaerobes are susceptible to clindamycin, and non-β-lactamase-producing strains of many anaerobes are susceptible to ampicillin (18–20). The ability of these two antibiotics to perturb the integrity of the intestinal flora of laboratory animals and humans is well documented (21–24). Thus, SB destruction appears to be linked to the presence of anaerobes in the gastrointestinal tract.

This study shows that ampicillin and clindamycin have a significant effect on the recovery and kinetics of SB in feces. Since SB use is predicated on a prior disturbance of gastrointestinal microflora by antibiotics, rational use of the yeast requires that this phenomenon be further investigated in humans.

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