Recovery and Elimination of the Biotherapeutic Agent, *Saccharomyces boulardii*, in Healthy Human Volunteers

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Saccharomyces boulardii (Sb) is a nonpathogenic yeast used to treat intestinal illnesses such as pseudomembranous colitis and antibiotic associated diarrhea. The behavior of this biotherapeutic agent in humans was determined (1) in investigating the effect of dose on the steady-state level and recovery and (2) in quantitating the effect of ampicillin on the recovery and elimination profile. As the Sb dose increased, the mean steady-state concentration of Sb increased significantly. The percentage recovery was dose independent. When a single Sb dose was administered 24 hr after beginning a course of ampicillin, there was a significant increase ($P < 0.01$) in both the area under the concentration versus time curve and the maximum fecal concentration compared to values obtained without ampicillin. Ampicillin increased steady-state recovery of the drug about twofold ($P < 0.05$) and steady-state levels about 2.4 times ($P < 0.01$). These studies have shown that there is a relationship between the dose and the amount of Sb recovered and that perturbation of the GI flora by ampicillin increases steady-state levels of Sb.

KEY WORDS: *Saccharomyces boulardii;* antibiotics; gastrointestinal kinetics; dose; recovery; biotherapeutic agent.

INTRODUCTION

Biotherapeutic agents, sometimes referred to as "probiotics" (1) or "bacteriotherapeutic agents" (2), are living microorganisms that have important therapeutic applications. A special strain of *Saccharomyces cerevisiae* has been used in the treatment of congenital sucrase-isomaltase deficiency (3); *Lactobacillus GG* (4), a nontoxigenic strain of *Clostridium difficile* (2), and *Saccharomyces boulardii* (5,6) have been used to treat pseudomembranous colitis in humans. Even though these unconventional agents have shown promise in the treatment of gastrointestinal diseases, very little attention has been directed toward defining the *in vivo* fate of orally administered microorganisms. Other fundamental questions which have not been addressed include the relationship between effect and fecal concentration, factors controlling the time course in the gastrointestinal tract, and drug interactions.

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Previously, daily oral administration of Sb (1.0 g) to healthy volunteers was found to achieve a steady-state concentration of 10^8 colony forming units (CFU)/g stool by day 3 (7). Less than 1% of an oral dose was recovered in the feces at steady-state concentrations. A recent study in rats examined the effect of antibiotics on the recovery of Sb and found that ampicillin produced a sevenfold increase in fecal recovery of Sb compared to controls, while neomycin appeared to have no effect (8). These data suggested that the fate of Sb may depend on the composition of the gastrointestinal flora. In this paper, the effect of dose on the kinetic profile and recovery of Sb was studied in healthy volunteers. In addition, the effect of ampicillin on the disposition of Sb in humans was investigated.

MATERIALS AND METHODS

Study Protocols

All of the studies were carried out in consenting healthy human volunteers using protocols approved by the Human Subjects Review Committee of the University of Washington. Three study protocols were employed. The first was used to determine the effect of Sb dose on recovery in stool. Inclusion criteria for selection were being 18–55 years old, having no history of chronic disease, having no diarrhea 4 weeks prior to the start of the study, being a nonsmoker, and having a "normal" daily bowel movement (i.e., one bowel movement per day). Exclusion criteria were an allergy to yeast, concurrent therapy with medications which affect the gastrointestinal tract, concurrent oral antifungal therapy, or the presence of $>10^6$ CFU yeast in stool as part of normal flora (pretreatment). Three doses of Sb were selected—0.1, 0.5, and 1.5 g—taken at 9 AM and 9 PM. Capsules containing 50 mg lyophilized Sb were used for the 0.2-g/day dose; otherwise, 250-mg capsules were taken. Each volunteer sequentially took each dose level of yeast for 1 week, separated by a 1-week drug washout period. The order of the doses was randomized. At the end of the rest week, volunteers were screened for the presence of Sb in stools. In all cases no Sb was present. During each week when Sb was administered, total 24-hr stool collections were made for 5 days beginning 48 hr after taking the first dose of Sb.

The second study protocol tested the effect of ampicillin on single-dose Sb elimination kinetics. The inclusion and exclusion criteria for the study were as described above except that volunteers were excluded if they had taken antibiotics within 8 weeks prior to study initiation and if they were female (there is a small risk of *Candida* vaginal infections with ampicillin ingestion). Volunteers were also not eligible if they were allergic to β-lactam antibiotics, had no history of penicillin use, or had abnormal blood urea nitrogen or creatinine levels. On Day 1, the volunteers took a single oral dose of Sb (four 250-mg capsules) in the morning. Total 24-hr stool collections were obtained for 7 days, followed by 7 days of a drug-free washout. After the washout period, ampicillin was administered for 7 days at a dose of 250 mg four times a day. Twenty-four hours after taking the first dose of ampicillin, a single 1.0-g dose of Sb was taken followed by total 24-hr stool collections for 7 days.

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The third study protocol tested ampicillin's effect on Sb elimination at steady state. Exclusion and inclusion criteria were as described above for the single-dose protocol. The experimental procedure differed from the single-dose protocol above only in that the 0.5 g of Sb was taken twice daily for 7 days.

Microbiologic Assays

Capsules containing 50 or 250 mg of lyophilized Sb were provided by Laboratoires Biocodex, Montrouge, France. Each stool sample was stored by the volunteer in a refrigerator and submitted for analysis within 12 hr. The stool was diluted 1:5 with 0.9% NaCl and homogenized in a blender. Concentrations of Sb were expressed as CFU per fecal sample and were determined by a modification of a procedure previously described (9). The limit of detection for Sb using this procedure was 10^3 CFU/g wet weight of stool. Serial 10-fold dilutions were carried out in phosphate-buffered 0.9% NaCl (pH 7). Dilutions were plated in triplicate on Sabouraud agar plates containing 1.0 mg/L ampicillin/sulbactam (Unasyn, Roerig, New York) and 1.0 mg/L gentamycin (Sigma, St. Louis, MO). Colonies were counted after a 48- to 72-hr incubation at 37°C. The identification of Sb colonies was confirmed by obtaining a negative germ tube test and by determining a characteristic metabolic sugar utilization pattern on API 20C strips. *Candida albicans* and *Saccharomyces cerevisiae* were detectable at low levels ($<10^6$ CFU) in some fecal samples. In a subsample of volunteers ($n = 5$), the effect of Sb on normal colonic flora was examined by quantitating the stool levels of selected groups of organisms before (preenrollment) and after Sb ingestion (4–5 days after taking 1.0 g of Sb daily). One gram of stool was diluted (1:5) in prereduced, supplemented peptone broth (Beckton Dickinson, Rutherford, NJ) and serially diluted in the assay described above except that prereduced peptone broth was substituted for saline. Culture media used included blood agar (total aerobes and total anaerobes), MacConkey agar (coliforms), *Brucella* agar (Bacteriodes), and phenylanaline agar (Clostridial species).

Statistical Analysis

Differences between mean values were determined using Student's t test. The mean values of paired differences for Sb were tested using the paired t test at a significance level of $P = 0.05$.

RESULTS

Dose–Response

Eight healthy volunteers (two female, six male) were given Sb at daily doses of 0.2, 1.0, and 3.0 g. Steady-state fecal levels of Sb were achieved by 72 hr (data not shown) for each dose. There was a significant trend of increased Sb levels in the stool as the administered oral dose of Sb was increased ($r^2 = 0.99$, $P < 0.05$). When the daily dose was increased from 0.2 to 1.0 or 3.0 g, there was a significant increase in mean Sb concentrations ($P < 0.05$) (Table I). There was no significant difference in the mean steady-state levels of Sb comparing the 1.0- and 3.0-g doses.

Table I. Dose Recovery of *Saccharomyces boulardii* in Adult Volunteers at Steady-State Concentrations

Sb dose (g)	n	Mean stool CFU ± SE ($\times 10^7$)	P value
0.2	8	3.66 ± 1.44	—[a]
1.0	8	17.5 ± 8.67	<0.01
3.0	7	86.1 ± 74.1	<0.05

[a] Two hundred milligrams used as baseline. P value from paired t tests.

When the total recovered CFU for Sb was determined between 72 and 120 hr for all volunteers, the amount recovered was also found to increase with an increase in dose (Fig. 1). The increase was significant ($P < 0.05$) comparing 0.2 versus 1.0 g but was not significant comparing 0.2 versus 3.0 g ($P > 0.05$). The greater intersubject variation at 3.0 g contributed to a loss of power to detect a significant difference (only 3% power). The mean (±SE) percentage steady-state recovery values for Sb measured between 72 and 120 hr at each dose were as follows: at 0.2 g, 3.86 ± 1.40; at 1.0 g, 2.44 ± 1.13; and at 3.0 g, 4.72 ± 4.08. These differences by dose were not significant.

In a subset of volunteers ($n = 5$), exposure to Sb did not significantly alter the quantitative populations of tested normal flora. The only substantive increases observed were for total aerobes ($1.4 \times 10^6 \pm 2.2 \times 10^6$ pre-Sb versus $2.1 \times 10^8 \pm 5.0 \times 10^2$ post-Sb) and total coliforms ($1.8 \times 10^6 \pm 3.8 \times 10^6$ pre-Sb versus $1.9 \times 10^7 \pm 35$ post-Sb) but these increases were not significant ($P = 0.14$ and $P = 0.09$, respectively).

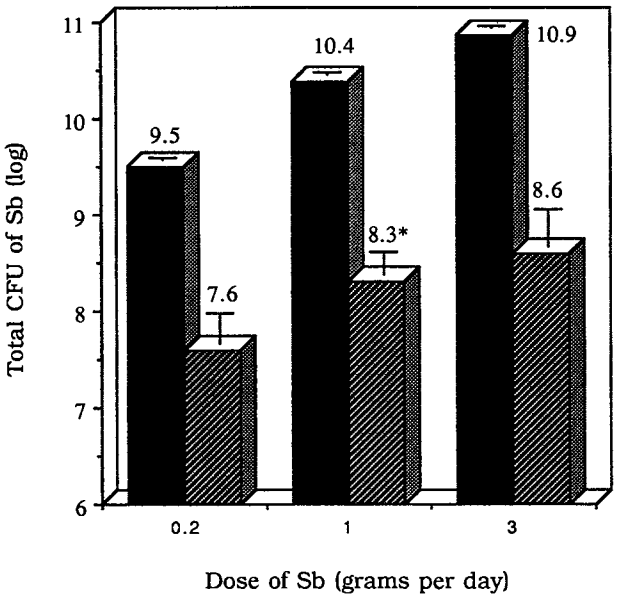

Fig. 1. The total CFU of Sb measured in stools collected between 72 and 120 hr after daily administration of Sb in the dose range of 0.2, 1.0, and 3.0 g per day. The filled columns represent the total CFU of viable yeast administered to each volunteer and the hatched columns indicate the recovered CFU (mean ± SE; $n = 8$ except for the 3-g dose, where $n = 7$). (*)$P < 0.05$ comparing the recovered CFU at 0.2 versus 1.0 g.

Single-Dose Kinetics

Ten volunteers completed the single-Sb dose study examining the influence of ampicillin on Sb elimination. The dose of Sb used was 1.0 g, chosen since this dose has been used in recent clinical efficacy trials (5,10). Ampicillin significantly increased the mean area under the concentration (AUC) versus time curve (Table II) (mean of 3.78×10^9 without ampicillin and 6.63×10^{10} with ampicillin; $P < 0.01$). Ampicillin exposure also significantly increased the mean maximum fecal concentration (C_{max}) for Sb (mean of 1.69×10^8 without ampicillin and 2.08×10^9 with ampicillin; $P < 0.01$). The fecal recovery of Sb was 0.12 ± 0.04% in the absence of ampicillin and 2.77 ± 1.99% in the presence of ampicillin, but this difference was not significant (Table II).

Steady-State Kinetics

Six volunteers completed the ampicillin study with the Sb steady-state protocol. The mean fecal output of Sb (Table III) measured between 72 and 120 hr (when steady state was achieved) in the absence of ampicillin was 2.58×10^8. The concentration of Sb was significantly increased in the presence of ampicillin (mean, 6.11×10^8; $P < 0.01$). The steady-state percentage recovery was increased about twofold with ampicillin ingestion ($P < 0.05$).

To examine intrasubject variability in the mean steady-state levels of Sb with repeat experiments, the steady-state yeast levels for five volunteers who had taken daily 1.0-g doses of Sb in at least two separate experiments are shown in Fig. 2. With the exception of subjects 8 and 10, the mean steady-state levels of Sb remained similar on an intravolunteer basis.

DISCUSSION

There are relatively few pharmaceuticals classified as living microorganisms that have proven therapeutic effects when administered to humans. Of those biotherapeutics which have been used in therapy, fundamental questions on the disposition kinetics of the agents in humans have not been fully addressed. When considering the mass balance of Sb after oral administration to healthy volunteers, the mean recovery in stool was found to be <5%, which was independent of the dose administered or whether it was administered as a single dose or daily to achieve steady-state concentrations. The relatively low recovery reported here is in agreement with the findings of Blehaut *et al.* (7), who, in addition, were also able to account for the majority of the dose administered being recovered as dead cells. A study in rats given both Sb and the antibiotic, ampicillin, has shown that Sb recovery is increased sevenfold in the presence of ampicillin, suggesting that the fate of Sb appears to lie in the composition of the gut flora (8). Ampicillin has been shown to disturb normal colonic flora (11), and when Sb and ampicillin were given concurrently, the recover of Sb at steady state was found to increase significantly. Since yeasts are resistant to antibacterials, this increase lends further support to the hypothesis that the human GI tract may also possess a group or groups of microorganisms that can "metabolize" or destroy the yeast. It is important to bear in mind that even with ampicillin treatment, much of the Sb dose is destroyed during passage through the GI tract. Either ampicillin was not able to block destructive microbial metabolism of Sb completely or other physiological processes (e.g., bile acid production, pancreatic proteases) are important toward Sb recovery. Quantitation of the relative importance of all processes involved in Sb destruction will require further study.

A recent human study using *Saccharomyces cerevisiae* reported maximal fecal counts of 10^5 CFU/g were reached with daily ingestion of 3×10^8 cells, and the yeast level decreased immediately once its administration had stopped (12). These investigators do not report a steady-state percentage recovery value, but if the assumption is made that the 10^5 CFU/g stool weight measurement is based on a stool weight of 200 g, this would correspond to about a 2% steady state recovery. Goldin *et al.* (13) studied survival of *Lactobacillus GG* in human volunteers and reported steady-state levels of about 10^6 CFU/g following daily doses of 4×10^{10}

Table II. The Effect of Ampicillin (Amp) on AUC, C_{max}, and Percentage Recovery of Sb After the Single-Dose Administration of 1.0 g Sb

Subject	AUC[a] (CFU/10^6)		C_{max}[b] (CFU/10^6)		% recovery	
	−Amp	+Amp	−Amp	+Amp	−Amp	+Amp
1	177	6,689	7.7	209	0.005	0.21
2	4,025	408,745	337	13,700	0.26	20.5
4	460	36,108	23.2	935	0.01	0.84
6	3,571	91,045	185	2,161	0.12	2.36
7	3,432	9,044	167	229	0.13	0.37
10	608	58,680	21	1,867	0.21	0.17
13	408	25,811	23.5	887	0.01	0.01
8	8,358	6,242	324	172	0.01	1.82
9	262	253	11.9	8.6	0.02	1.01
14	16,553	20,680	592	667	0.37	0.40
Mean ± SEM (CFU × 10^6)	3,785 ± 1,640	66,330 ± 39,069	169 ± 62	2,084 ± 1,311	0.12 ± 0.04	2.77 ± 1.99
Significance	$t = 4.2$, $P < 0.01$		$t = 3.3$, $P < 0.01$		$t = 1.3$, $P > 0.05$[c]	

[a] Area under the concentration versus time curve.
[b] Maximum concentration of Sb measured in the stool sample (CFU).
[c] Not significant.

Table III. The Effect of Ampicillin (Amp) on the Mean CFU of Sb Measured at Steady-State Concentrations After the Daily Administration of 1.0 g Sb

Subject	Mean CFU ± SEM (÷10^6)		% recovery	
	−Amp	+Amp	−Amp	+Amp
1	105 ± 1.00	57.3 ± 30.4	0.06	0.03
7	351 ± 117	629 ± 143	0.35	0.50
8	483 ± 143	1070 ± 199	0.29	0.64
4	28.9 ± 13	246 ± 48	0.02	0.15
6	576 ± 57	1347 ± 197	0.46	1.08
10	6.5 ± 3.6	315 ± 167	0.004	0.19
Mean ± SEM	258 ± 100	611 ± 207	0.20 ± 0.08	0.43 ± 0.16
Significance	$t = 6.67, P < 0.01$		$t = 2.61, P < 0.05$	

or 2 × 10^{11} CFU. This would represent a recovery of <1% assuming a 200-g daily stool output. The low fecal recovery of *Saccharomyces cerevisiae* and *Lactobacillus GG* is consistent with the findings reported in this paper for Sb.

There was considerable intersubject variability in the levels of Sb measured in stool especially at the highest dose tested. Consistency in the Sb levels achieved upon repeated administration to the same individual was observed, however. Preliminary experiments with the nonmetabolizable marker, polyethylene glycol 4000, indicate that intersubject variation in Sb recovery cannot be attributed to differences in transit time or other factors affecting marker recovery (Klein *et al.* unpublished observations).

Therapeutic effects related to fecal concentrations of Sb have not been addressed in the literature. A threshold level of the yeast in the GI tract may be necessary. It could be postulated that individuals that have low Sb intestinal levels may not respond as well to treatment as those individuals with high Sb levels. In the germ-free mouse model for pseudomembranous colitis, only doses which gave fecal Sb levels >5 × 10^6 CFU/g were protective (14). A dose–effect relationship has not been established for Sb in humans so far. In the present study, the lowest tested dose (0.2 g per day) gave steady-state fecal levels of 4 × 10^7 CFU, and the highest dose (3.0 g per day) gave levels of 9 × 10^8 CFU. One gram of Sb per day has been shown to reduce antibiotic associated diarrhea in hospitalized patients (10) and to decrease the incidence of pseudomembranous colitis relapse (5,6). In the clinical setting, where Sb would often be prescribed concurrently with antibiotics, the level of surviving Sb could be expected to increase two- to threefold during therapy with antibiotics like ampicillin.

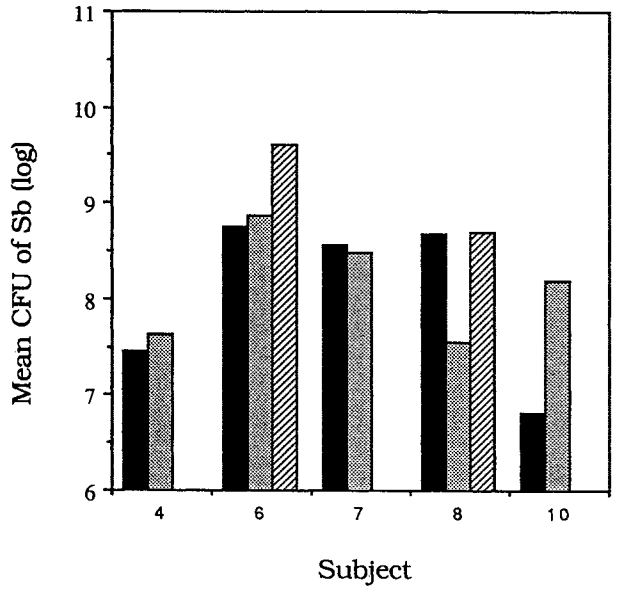

Fig. 2. Mean steady-state levels (72–120 hr) of Sb measured in the same five volunteers in at least two separate experiments. The Sb dose was 1.0 g daily. Filled columns represent steady-state CFU in the first experiment, shaded columns in the second experiment, and hatched columns in the third experiment (subjects 6 and 8 only).

With the increased interest in biotherapeutic agents (treatment for diarrhea, future use for orally administered recombinant strains, etc.), there is a need for methods/approaches to study the *in vivo* behavior of biotherapeutic agents. Sb can also serve as a reference or model since its measurement in stool is relatively straightforward and because widespread clinical use has provided confidence with the issue of its human safety.

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