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Prevention of ß-Lactam-Associated Diarrhea by *Saccharomyces boulardii* Compared with Placebo

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***Objectives*: To determine the safety and efficacy of a new preventive agent for antibiotic-associated diarrhea (AAD) in patients receiving at least one ß-lactam antibiotic. *Methods*: A double-blinded, placebo-controlled, parallel group study was performed in a high-risk group of hospitalized patients receiving a new prescription for a ß-lactam antibiotic and having no acute diarrhea on enrollment. Lyophilized *Saccharomyces boulardii* or placebo (1 g/day) was given within 72 h of the start of the antibiotic(s) and continued until 3 days after the antibiotic was discontinued, after which the patients were followed for 7 wk. *Results*: Of the 193 eligible patients, significantly fewer, 7/97 (7.2%), patients receiving *S. boulardii* developed AAD compared with 14/96 (14.6%) on placebo ($p = 0.02$). The efficacy of *S. boulardii* for the prevention of AAD was 51 %. Using a multivariate model to adjust for two independent risk factors for AAD (age and days of cephalosporin use), the adjusted relative risk was significantly protective for *S. boulardii* (RR = 0.29, 95% CI = 0.08, 0.98). *Conclusion*: The prophylactic use of *S. boulardii* given with a ß-lactam antibiotic resulted in a significant reduction of AAD with no serious adverse reactions.**

INTRODUCTION

Although many newly developed antibiotics have broad spectrums of activity and fewer side effects, antibiotic-associated diarrhea (AAD) remains a common complication. The incidence of AAD has ranged from 3.2 to 29/100 in studies of hospitalized patients (1-3). The onset of AAD may be rapid (while the patient is on antibiotics) or may be delayed for up to 6 wk after the antibiotics have been discontinued (4, 5). The severity of AAD may range from uncomplicated to severe diarrhea and serious complications may arise, including electrolyte imbalances, dehydration, pseudomembranous colitis, toxic megacolon, or death (1, 2, 6). The occurrence of AAD in hospitalized patients has also been associated with increased length-of-stays by 8-20 days, higher medical care costs, five-fold increased risks of developing other nosocomial infections, and three-fold increases in mortality (3, 6-8). Identified etiologies of AAD include *Clostridium difficile* (25-30%), and to a lesser extent, *Salmonella*, *C. albicans*, and enterotoxigenic *Clostridium perfringens*; but the etiologies of the majority of AAD remain unclear (1, 4, 9). Speculation on the causes of AAD include: *1)* the overgrowth of *C. difficile*; *2)* the unveiling of toxin receptors or attachment sites caused by the disappearance of the normal flora; *3)* the decrease in short-chain fatty acids due to the loss of bacterial strains responsible for the metabolism of complex carbohydrates; or *4)* the lack of nutrient competition caused by changes in normal flora (10-14). The various theories of the etiology of AAD are tied together by the common impact of antibiotics on the normal colonic flora. Previous studies have found that neither the dose nor duration of antibiotic increases the risk of AAD, but the type of antibiotic may be important (1, 3, 15). Antibiotics with a spectrum of activity that includes anaerobic bacteria (especially cephalosporins, penicillins, or clindamycin) have been associated with higher rates of AAD, although nearly all types of antibiotics have been implicated (1, 16-21). The most frequently implicated broad spectrum antibiotics are those agents that impact the anaerobic component of the fecal flora; consequently, ß-lactam antibiotics have been found to have the highest frequencies of AAD (1, 2, 16, 22).

Treatment modalities for AAD are limited because no established treatment exists for non-*C. difficile*-associated AAD except for discontinuing the inciting antibiotic and supportive care (1). Treatment for *C. difficile*-associated AAD may require oral metronidazole or vancomycin, but 20% of the patients may develop subsequent recurrences after antibiotic treatment ceases (4).

Saccharomyces boulardii is a nonpathogenic yeast that has been used in Europe as an anti-diarrheal agent (23, 24). Results from *in vivo* studies have shown that *S. boulardii* reaches high, steady state levels in the Stool (10^7-10^8) within 3-5 days and is no longer detectable by 2-6 days after discontinuation of the yeast (25-27). *S. boulardii* has been shown to be effective in the treatment of *C. difficile* colitis and, in one study

by Surawicz *et al*, *S. boulardii* was effective in preventing AAD in patients with a wide variety of types of antibiotic exposure (28-30). Because of the frequent use of ß-lactam antibiotics and the high risk of AAD associated with these types of antibiotics, we performed a double-blinded, placebo-controlled trial of *S. boulardii* in patients receiving at least one type of ß-lactam for the prevention of AAD.

MATERIALS AND METHODS

Patient population

Consecutive adult inpatients receiving new prescriptions for at least one ß-lactam antibiotic were screened at one of four hospitals: University of Washington Medical Center and Harborview Medical Center, Seattle, Washington; University of Kentucky Medical Center, Lexington, Kentucky and St. Louis University Medical Center, St. Louis, Missouri. The Human Subjects Review Committee at each center approved the study protocol, and each patient gave written informed consent. All patients were adult (18-86 yr) inpatients who received a new prescription of a ß-lactam antibiotic, either alone or with another antibiotic, for at least 48 h and had no diarrhea less than 24 h after enrollment. In the case of different antibiotics given sequentially, patients were eligible if the antibiotics were started less than 7 days before enrollment, and the time between antibiotic courses (one being a ß-lactam) was less than 48 h. ß-lactam antibiotics included medium-to-broad spectrum penicillins, combination penicillins (penicillins with a ß-lactamase inhibitor), or any cephalosporin. Patients receiving only penicillin G or penicillin V (narrow spectrum penicillins) were not eligible for the trial.

Patients were assigned to either oral *S. boulardii* or placebo at 1 g (3 X 10^{10} colony-forming units) per day (2 250-mg capsules, twice a day). The study drug assignment was randomized within three age groups for each center (aged 18-44, 45-69, or 70-99). The appearance and odor of the capsules of the patented *S. boulardii* and placebo were identical. The 1:1 (*S. boulardii*:placebo) randomization and packaging of the blinded study kits was performed at Laboratoires Biocodex (Montrouge, France) to ensure that the study investigators did not have access to the identity of the study drug. The study drug was started within 72 h of the ß-lactam antibiotic and continued for 3 days after the antibiotic was discontinued. The maximum duration of study drug treatment was 28 days. After the discontinuation of the study drug, the patients were followed for a total of 7 wk, which is 1 wk longer than the mean incubation period for AAD quoted in the literature (4, 5). During follow-up, data was collected on clinical symptoms and delayed adverse reactions, which included physical symptoms, fever, rash, changes in blood chemistries, urinary indicators (protein, BUN, glucose), or changes in liver enzymes. The patients were given a standardized daily diary to record stool frequency and consistency, antibiotics, other medications taken, and any adverse reactions. The patients were also followed by study investigators daily while hospitalized and were phoned weekly after discharge.

Case definitions

An eligible antibiotic was an antibiotic given for at least 48 h by an oral or intravenous route. Multiple antibiotics were defined as antibiotics given either simultaneously with the ß-lactam antibiotic or sequentially (with a maximum of 48 h between the last dose of the first antibiotic and the start of the second antibiotic).

Diarrhea was defined as a change in bowel habit with at least 3 loose stools/day for at least 2 consecutive days. AAD was defined as diarrhea associated with at least one ß-lactam antibiotic with no other etiology of diarrhea identified (medications, lactose intolerance, nasogastric tube feedings, enemas). The etiology of all cases of diarrhea was determined independently by three blinded investigators. Study drug failure was defined as a patient developing AAD either while on the antibiotic or at any time during the 7 wk of follow-up after the study drug was discontinued. Study termination was either by completion of the study or by censoring because of: refusal, death, initiation of a new antibiotic while no longer on study drug, attrition, or the initiation of an exclusion drug (oral antifungal).

A modified standard index (APACHE) was used to quantitate the patient's basic health status to stratify acutely ill patients (31). The modified APACHE index substituted oral for rectal temperature, SGOT, SGPT, total protein, and serum glucose for respiratory data, arterial pH, serum sodium, and serum potassium. This APACHE index was verified on a separate data base, in which 144 patients had complete blood chemistries, and was found to have a mean score of 3.9 ± 2.5 for patients with mild underlying disease and a significantly higher score (mean = 5.9 ± 2.9, t = 4.5, $p < 0.001$) for patients with more severe underlying disease conditions (28).

Antibiotic prescription patterns

To determine the trends of antibiotic use at two of the study hospitals (Harborview Medical Center and University of Washington Medical Center), antibiotic purchase inventories were collected from the pharmacies, and the total units used were calculated from cost per unit and total annual cost for each type of antibiotic from 1989-1992 where data was available.

Microbiological methods

Stool samples or rectal swabs were collected on enrollment, at the end of study drug treatment, and at any time that diarrhea occurred. Inoculum from stool or rectal swabs was plated onto Difficile agar plates (Prepared Media Laboratories, Tualatin, Oregon) or CCFA (BBC Laboratories) and incubated anaerobically for 48 h at 37°C. To facilitate detection of *C. difficile* at low levels, an aliquot of stool was also inoculated

into prereduced supplemented peptone broth (Becton Dickinson Vacutainer System, Rutherford, NJ), containing 39 µg/ml cefoxitin and 0.1% pure sodium taurocholate, and incubated for 72 h at 37°C. *C. difficile* was identified using standard procedures (32). Stools were also tested for cytotoxin within 48 h of collection using CHO cell tissue cultures. Diluted stool (1:1) was centrifuged (3000 RPM for 10 min) and filtered (0.8 µl pore size). Serially diluted filtered stool was then added to cell cultures and observed for cytopathic effect at 24 h. The specificity of the cytopathic effect was checked by neutralization using *Clostridium sordellii* anti-toxin (33).

Statistical methods

The patients were evaluated on an intention-to-treat basis to provide a more valid assessment of treatment efficacy in routine clinical practice (34). All patients were included in the trial including completed and censored patients (owing to attrition, death, refusal, or initiation of exclusion drugs). Differences between means were assessed using the Student's *t* test, differences between group proportions were assessed using the χ^2 statistic or, if the sample size was small, Fisher's exact test. Nonparametric data was analyzed using the Wilcoxon ranked sum test. To test the hypothesis that the incidence of AAD was decreased in the patients receiving *S. boulardii* compared with the incidence of AAD in patients receiving placebo, a binomial exact test was used to determine a *p* value (35). Two-tailed tests were used to test the significance at a $p \leq 0.05$ level for factors that were not known *a priori* to increase or decrease the incidence of AAD. The efficacy was determined using the equation: $[(I_p-I_t)/I_p]$ 100, where I_p is the incidence of AAD in the patients receiving placebo and I_t is the incidence of AAD in patients receiving *S. boulardii*. Unadjusted relative risks were calculated from the formula $(1_t/I_p)$, and 95% confidence intervals were calculated (36). Failure curves were calculated by the Kaplan-Meier method, with data stratified according to treatment group and compared with the Mantel log-rank test. Logistic regression analysis was used to assess the relation between AAD and treatment while simultaneously controlling for other possible risk factors of AAD. Regression variables were fitted by a nested hierarchy approach using EGRET software (Statistics & Epidemiology Research Corporation, Seattle, Washington). Coefficients of the regression variables were tested for significance using differences of log likelihood statistics interpreted as χ^2.

RESULTS

Enrollment

During the study enrollment period (March 1989-December 1992), 12,546 patients were screened for entry. Reasons for noninclusion included: antibiotic started >72 h of interview (24%), immunosuppression (15%), antibiotic given <48 h (10%), catastrophic illness (9%), no telephone (7%), discharged before interview (6%, < 18 yr old (4%), on oral anti-fungal medication (4%), refused participation (2%), and miscellaneous (19%). Of the 208 patients enrolled in the study, 15 were ineligible for the following reasons: study drug was initiated >72 h after the antibiotic(s) were begun (n = 9), oral anti-fungal drug started < 48 h from enrollment (n = 2), diarrhea < 24 h after enrollment (n = 1), and antibiotic or study drug given for < 48 h (n = 3). To determine if selection bias may have occurred, a comparison of the ineligible patients with the eligible patients was performed; there were no significant differences by age, gender, APACHE score, number of antibiotics or medications on enrollment, reasons for antibiotic prescription, or assignment to *S. boulardii* or placebo (data not shown).

Of the 193 eligible patients, 129 (67%) completed the trial, 25 (13%) were censored during the study drug period, and 39 (20%) were censored during the 7-wk follow-up period. Of the 64 censored patients, 28 were lost to follow-up, 27 received a new antibiotic prescription poststudy drug, four developed adverse reactions (nausea or constipation), three died, and two received oral nystatin. A comparison of the 129 completed patients with the 64 censored patients revealed no significant differences by study drug assignment, gender, age, antibiotic use, enrollment site, or APACHE score (data not shown). Censored patients had significantly shorter mean follow-up times than completed patients (19 ± 2 *vs* 55 ± 20 days, respectively, $t = 14.7$, $p < 0.001$), received less total g of study drug (12.4 ± 8.1 *vs* 16.0 ± 7.5 g, respectively, $t = 3.1$, $p < 0.01$), and developed less AAD (4.7% *vs* 14.0%, respectively, Fisher's $p = 0.04$), but none of these factors were significantly different by the type of study drug received (*S. boulardii* or placebo) (data not shown).

Treatment groups

Of the 193 eligible patients, 97 were assigned to *S. boulardii* and 96 were assigned to placebo. To judge if bias was introduced by treatment assignment, a comparison of the group of patients receiving *S. boulardii* and the group receiving placebo was performed. No statistically significant differences were noted in the patients assigned to *S. boulardii* compared with patients given placebo (Table 1). Patients treated with *S. boulardii* received a mean of 2.4 ± 1.0 antibiotics, which was similar to the mean number of antibiotics (2.5 ± 1.1) received by patients on placebo. The types of antibiotics and medications were not significantly different in patients on *S. boulardii* or placebo (data not shown).

Site comparison

Because patients were enrolled at four hospitals, a comparison of the patients by enrollment site was performed (Table 2). Patients from the four sites were generally similar except for differences in age, number of medications, and APACHE index. Patients at St. Louis University were significantly older, had higher APACHE index scores, and received more antibiotics and medications; but none of these factors resulted in a higher frequency of AAD.

TABLE 1
Baseline Characteristics by Study Drug Group

	Saccharomyces boulardii (n = 97)	Placebo (n = 96)	*p* value
Age (Mean ± SD)	40.7 ± 16.0	42.3 ± 17.7	t = -0.65
			p = 0.51
Randomized age groups			
18-44	64 (66.0%)	62 (64.6%)	χ^2 = 0.27
45-69	25 (25.8%)	24 (25.0%)	df = 2
≥ 70	8 (8.2%)	10 (10.4%)	p = 0.87
Gender			
Male	62 (63.9%)	63 (65.6%)	χ^2 = 0.009
Female	35	33	df = 1
			p = 0.92
History of antibiotics* (Mean ± SD)	0.43 ± 0.68	0.35 ± 0.60	t = 0.86
			p = 0.39
History recent surgery	16 (16.7%)	23 (24.0%)	χ^2 = 1.16
			p = 0.28
APACHE (Mean ± SD)	7.96 ± 4.11	7.97 ± 4.68	t = -0.03
			p = 0.97
Number of medications (Mean ± SD)	6.9 ± 5.0	7.8 ± 5.8	t = -1.17
			p = 0.24
Number of antibiotics (Mean ± SD)	2.4 ± 1.0	2.5 ± 1.1	t = -0.65
			p = 0.52

* Any antibiotics given 6 wk before eligibility antibiotics.

TABLE 2
Comparison of Study Variables by Enrollment Site

	HMC (n = 79)	UH (n = 13)	St. Louis (n = 39)	Kentucky (n = 62)
Study drug				
S. boulardii	41 (51.9%)	6 (46.2%)	19 (48.7%)	31 (50%)
Placebo	38 (48.1%)	7 (53.8%)	20 (51.3%)	31 (50%)
Outcome				
AAD	12 (15.2%)	3 (23.1%)	3 (7.7%)	3 (4.8%)*
No AAD	67	10	36	59
Age (Mean ± SD)	38.2 ± 14.4	32.5 ± 14.8	47.8 ± 18.5*	43.7 ± 17.7*
APACHE (Mean ± SD)	6.6 ± 3.3	6.5 ± 3.4	10.4 ± 5.0*	8.3 ± 4.6*
N antibiotics (Mean ± SD)	2.2 ± 1.0	2.1 ± 1.1	2.8 ± 0.8*	2.5 ± 1.1
N medications (Mean ± SD)	6.9 ± 4.3	5.5 ± 5.2	9.4 ± 5.0*	7.0 ± 6.2
C. difficile				
Positive	19 (24%)	5 (38%)	0	0*
Negative	60	8	2	39
Follow-up (Mean days ± SD)	53.6 ± 27.5	49.9 ± 23.0	48.2 ± 26.7	45.7 ± 24.2

* $p < 0.05$ compared with HMC as base line.
HMC, Harborview Medical Center; UH, University Hospital; St. Louis, St. Louis University Medical Center; Kentucky, University of Kentucky Medical Center.

Patients at the University of Kentucky had a significantly lower frequency of AAD, were older, had higher APACHE scores, and had significantly lower frequency of *C. difficile* (Table 2).

Frequency of AAD

Of the 193 eligible patients, 21 (10.9%) experienced AAD during the study period. Seven patients reported diarrhea that did not meet the case definition of AAD. Blinded assessment assigned the cause of nonantibiotic-associated diarrhea to: nasogastric tube feeding (n = 3), laxative use (n = 2), lactose intolerance (n = 1), and viral gastroenteritis (n = 1).

The mean incubation period of AAD (from the first day of the antibiotic to the first day of diarrhea) was 18 days and ranged from 3 to 56 days. The severity of AAD was measured by the duration of AAD (which ranged from 2-25 days) and stool frequency (which ranged from 3 to 9 loose or watery stools/day) and the presence of fever (oral ≥ 101°F) in 9.5% of the patients with AAD. In patients on placebo, the duration of AAD was shorter (median of 4 days) while patients were on antibiotics (which may have reflected the rapid response to discontinuation of the inciting antibiotic), but the duration was prolonged (rnedian of 18 days) for patients with delayed AAD (when discontinuation of the antibiotic is no longer a treatment option).

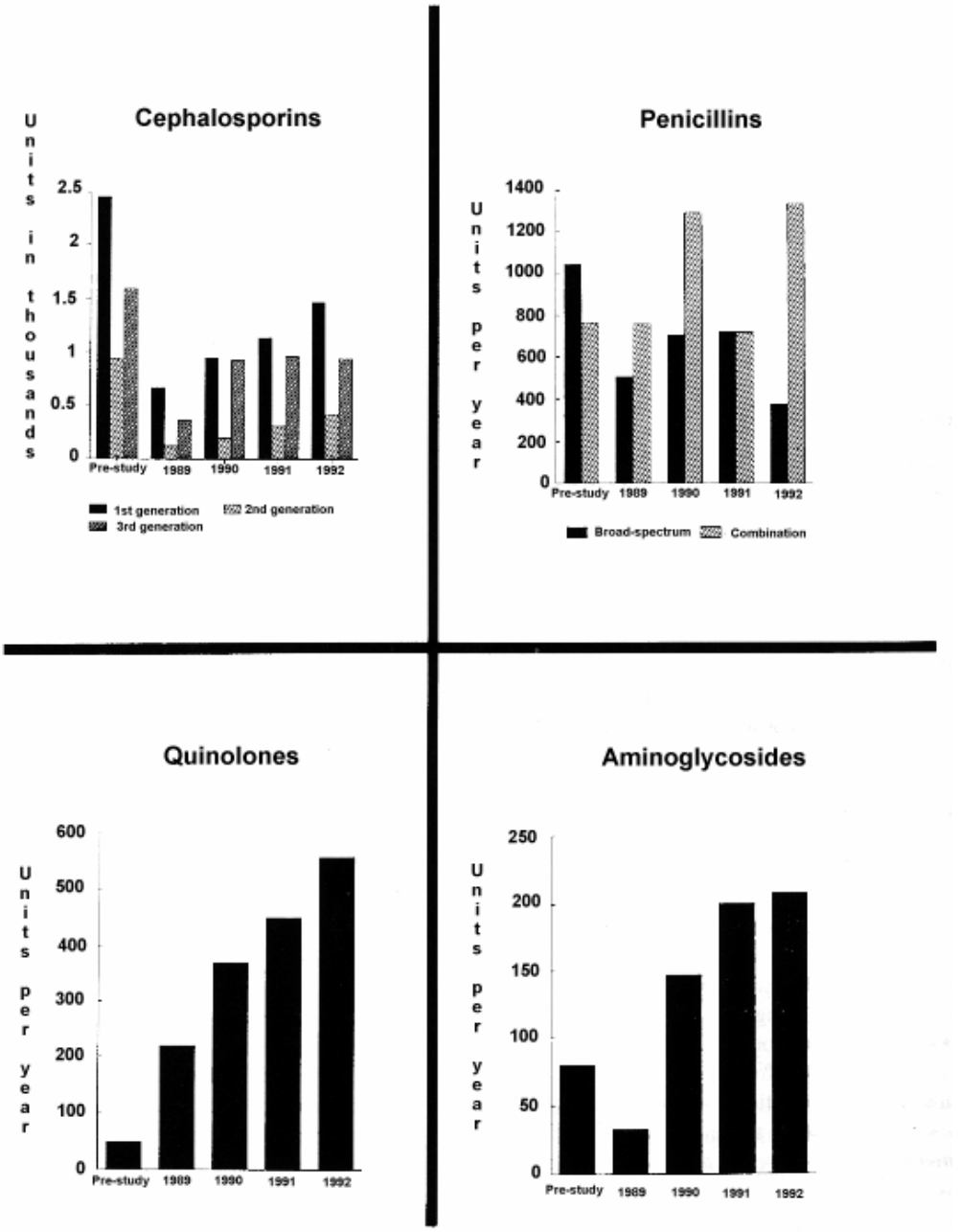

FIG. 1. Antibiotic-use patterns over time. Data was compiled from two study hospitals (Harborview Medical Center and University of Washington Medical Center, Seattle, Washington). Prestudy refers to data collected from the same data source from 1986 to 1989. Combination penicillins include penicillins with ß-lactamase inhibitors.

In patients on placebo, the frequency of AAD was higher for patients on multiple antibiotics (15%) than patients on a single ß-lactam (11.8%).

Antibiotic-use trends

Basic antibiotic-use trends were recorded at selected study hospitals during the period of patient enrollment to observe if time trends of antibiotic use were associated with the changing frequency of observed AAD (Fig. 1).

Use of first- and second-generation cephalosporins gradually increased, and third-generation cephalosporin use remained constant during the last 3 yr of the study. Broad-spectrum penicillin use decreased from 1988 to 1992, whereas the use of combination penicillins (those with ß-lactamase inhibitors) increased. The use of narrow-spectrum antibiotics (aminoglycosides) and other broad-spectrum antibiotics (quinolones) increased over the study years. The total number of prescribed antibiotic units generally

TABLE 3
Factors Associated with Antibiotic-Associated Diarrhea

Factor	Patients with AAD (n = 21)	Patients without AAD (n = 172)	Total (N = 193)	*p* value*
Study drug				
S. boulardii	7 (7.2%)	90	97	$p = 0.03$**
Placebo	14 (14.6%)	82	96	
C. difficile				
Positive	7 (29%)	17	24	$\chi^2 = 4.59$
Negative	11 (10%)	98	109	$p = 0.03$
Not determined	[3]	[57]		
Age blocks***				
18-44	18 (14.3%)	108	126	$\chi^2 = 5.46$
45-69	1 (2.0%)	48	49	$p = 0.06$
>69	2 (11.1%)	16	18	
Cephalosporin duration (Mean days ± SD)	10.3 ± 12.9	6.8 ± 7.4	—	$t = 1.77$
				$p = 0.08$
Antibiotic exposure				
Single ß-lactam	4 (11.4%)	31	35	Fisher's
Multiple	17 (10.8%)	141	158	$p = 0.55$

* *p* values from unadjusted estimators unless otherwise noted.
** *p* value from binomial probability.
*** Age within *a priori* randomized blocks.

remained stable from 1989 to 1993. The frequency of AAD in the total study population remained fairly constant: in 1989 (11.3%), 1990 (11.2%), 1991 (13.3%), and 1992 (8.7%), even though there were major changes in the types of antibiotics used by these hospitals over the same time period.

Risk factors for AAD

In unadjusted estimates of increased risk for AAD, two factors were found to be significantly associated with AAD (Table 3): *C. difficile* positivity and assignment to placebo. Two other factors showed a trend ($0.05<p<0.08$) of increasing the risk for AAD (age < 45 yr and increasing days of cephalosporin use). Stool *C. difficile* cytotoxin or culture assays were available from 133/193 (69%) of the eligible patients. In the 133 patients tested for *C. difficile*, 7/18 of the cases of AAD were associated with *C. difficile*. Of the 24 patients with positive *C. difficile* assay results, seven had AAD and 17 were asymptomatic carriers of *C. difficile*. The incidence of AAD was significantly greater in *C. difficile*-positive patients (7/24, 29.2%) than in *C. difficile*-negative patients (11/109, 10.1% $\chi^2 = 4.59$, $p = 0.03$). The unadjusted relative risk for *C. difficile* was 2.9 (95% CI = 1.25, 6.69).

The mean age of patients with or without AAD was not significantly different (37 ± 15 yr and 42 ± 17 yr, respectively), but there was a trend of patients with AAD being younger (18-44 yr old, $p = 0.06$). Because of the study design, all patients received high-risk ß-lactam antibiotics, and the comparison between penicillins and cephalosporins did not yield significant differences of AAD development. Patients with AAD did have a trend for longer exposures to cephalosporins (Table 3). Medical history variables (history of gastrointestinal surgery, smoking, alcohol abuse, allergies, or prior hospitalizations) were also similar in patients with AAD and without AAD (data not shown). Enrollment characteristics (gender, APACHE, type of primary infections, chronic conditions) and types of medications received during the study were not significantly associated with AAD (data not shown).

Efficacy of Saccharomyces boulardii for AAD

Seven (7.2%) of the 97 patients receiving *S. boulardii* developed AAD compared with a significantly higher frequency (14/96, 14.6%) of patients assigned to placebo ($p = 0.02$). Thus, the efficacy for *S. boulardii* in preventing AAD was 51%. The unadjusted relative risk of developing AAD for patients on *S. boulardii* compared with patients on placebo was 0.48 (95% CI = 0.23, 0.97). The characteristics of AAD in the two study groups is shown in Table 4. Of the seven patients who received *S. boulardii* and developed AAD, five developed AAD while on antibiotics, and the remaining two patients developed AAD during the follow-up period. Of the 14 patients on placebo, eight developed AAD while on antibiotics, and the other six had delayed AAD. Although the diarrhea severity (as measured by daily stool frequency) was not significantly different for the two groups, the duration of diarrhea was significantly less for patients on *S. boulardii* both while on antibiotics and postantibiotic AAD (Table 4). *S. boulardii* also appeared to slow the development of AAD, as reflected by longer incubation periods observed in the *S. boulardii*-treated patient group (Table 4).

The efficacy of *S. boulardii* was examined by the type and number of antibiotics received by the patients. There was no significant difference for AAD in the two study groups given single ß-lactams, but there was a trend for a lower frequency of AAD in patients on *S. boulardii* compared with placebo

TABLE 4
Characteristics of Antibiotic-Associated Diarrhea by Study Drug Group

Characteristic	*S. boulardii* (n = 7)	Placebo (n = 14)	*p* value
Number with AAD			
During antibiotic/study drug	5	8	Fisher's
Posttreatment (7-wk follow-up)	2	6	$p = 0.44$
Total	7	14	
Incubation period (Mean days ± SD)			
During antibiotic/study drug	11.2 ± 6.5	7.1 ± 7.2	$t = 4.18$
			$p < 0.01$
Posttreatment (7-wk follow-up)	28.5 ± 12.0	20.7 ± 14.9	$t = 4.04$
			$p < 0.01$
Duration of AAD (median day)			
During antibiotic/study drug	3.0	4.0	$t = 26.0$
			$p < 0.05$
Posttreatment (7-wk follow-up)	2.5	18.0	$t = 24.5$
			$p < 0.05$
Diarrhea severity (Mean stools/day ± SD)			
Total	4.9 ± 2.2	5.2 ± 1.2	$t = 1.2$, NS

NS, not significant.

receiving multiple antibiotics (6% *vs* 15%, respectively, $p = 0.06$). Of the 97 patients given a penicillin and at least one other antibiotic, the frequency of AAD was significantly lower (2.4%) in the 42 patients on *S. boulardii* compared with the 55 patients on placebo (16.4%, Fisher's $p = 0.02$). Of the 116 patients given a cephalosporin and at least one other antibiotic, the frequency of AAD was 6.9% in the 58 patients on *S. boulardii* and 15.5% in the 58 patients on placebo, but this difference was not significant (Fisher's $p = 0.12$).

In the 24 patients with positive *C. difficile* assays, the frequency of AAD was not significantly different by the type of study drug assignment; 3/10 of the patients on *S. boulardii* developed AAD compared with 4/14 on placebo. The power of detecting a significant difference based on the sample size of 24 and the above rates was less than 3%.

A multivariate unconditional logistic regression model was used to adjust for risk factors and for *a priori* randomized age blocks to obtain a risk estimate for the study drug independent of these factors. Two factors were found to be significant in the multivariate model: age and increasing days of cephalosporin use. The types, dose, duration, or number of other antibiotics were not significant after the two risk factors had been included in the model. The multivariate adjusted relative risk for the study drug showed significant protection against AAD by *S. boulardii* (RR = 0.29, 95% CI = 0.08, 0.98). The final model with the above factors was significantly predictive of AAD ($\chi^2 = 11.3$, $p = 0.02$). As shown on the Kaplan-Meier Curve (Fig. 2), by the end of the study significantly more patients receiving placebo developed AAD compared with patients receiving *S. boulardii* ($\chi^2 = 3.71$, $p = 0.05$).

To assess if there was a bias due to censoring of patients, the effectiveness of *S. boulardii* was compared in completed and censored patients. The multivariate adjusted relative risk of developing AAD for patients receiving *S. boulardii* remained significantly protective (RR = 0.33, 95% CI= 0.02, 0.67) when other factors were adjusted for (length of follow-up, dose of study drug, and censoring status).

Safety and adverse reactions

Of the 193 enrolled patients, 185 (96%) returned completed adverse reaction forms. There were no significant adverse reactions with the exception that placebo patients reported more intestinal gas (n = 7, 7.4%) than *S. boulardii*-treated patients (n = 0, $p = 0.01$) and significantly more patients given placebo reported fever (n = 5, 5.3%) compared with *S. boulardii*-treated patients (n = 0, $p = 0.04$).

DISCUSSION

Even with the advent of newer improved antibiotics, AAD remains a clinical concern. This trial focused on a high-risk group of patients, namely those receiving ß-lactam antibiotics, to test a new prophylactic agent for AAD, and *S. boulardii* was found to be effective. Previous trials with AAD have involved the treatment of acute AAD, but a prophylactic agent would be most beneficial because this would decrease the medical complications and cost of treating acute disease. The severity of AAD in this study was significant as judged by the duration of diarrhea (median 4.5 days, range 2-25 days), frequency (mean 5 stools/day), and presence of fever (9.5%). None of the patients with AAD had endoscopic examinations, so it is unknown if colitis or pseudomembranous colitis was present. However, in patients with *C. difficile* disease, the diarrhea was sufficiently severe in 57% of the patients to require treatment with vancomycin or metronidazole.

The frequency of AAD is dependent on several factors and may be high in some populations. The incidence in

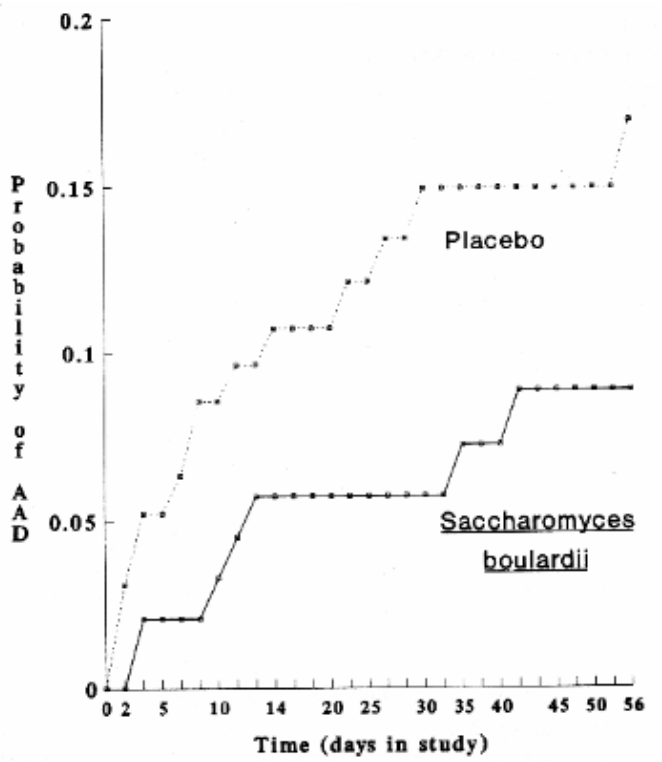

FIG. 2. Kaplan-Meier probability curve for the probability of developing AAD. The patients on *S. boulardii* (n = 97) are denoted by the *solid line*, and patients on placebo (n = 96) are denoted by the *dotted line*.

this study was not trivial (14.6 cases of AAD/100 enrolled patients on placebo). The rate of AAD at one of the study hospitals (U. of Kentucky) was lower despite an older, sicker study population, and this may have been due to the lower prevalence of *C. difficile*, the most common infectious etiology of AAD. The frequency of AAD in a specific institution may also vary over time because of factors such as changes in antibiotic prescribing patterns, patient demographies, or in-hospital infection control procedures (2, 3, 17). The incidence of AAD in patients (given placebo) at one institution (Harborview Medical Center) fell from 22% in a study conducted from 1986 to 1989 (19) to 16% in this study done from 1989 to 1992. This decrease in AAD was noted despite the observation that patients in the more recent study were sicker (mean APACHE of 8) compared with the patients in the earlier trial (mean APACHE of 5). Third-generation cephalosporin and broad-spectrum penicillin use decreased during this study (Fig. 1) whereas the use of aminoglycosides and quinolones increased. In other studies, increases in AAD incidence have also been associated with increases in third-generation cephalosporin use (17, 37, 38). Our hypothesis for the observed decreasing rate of AAD was the decrease in third-generation cephalosporins used during this study period.

In this study, *S. boulardii* was found to decrease the frequency of AAD by 51%. Of interest, *S. boulardii* not only decreased the number of patients developing AAD, but also significantly shortened the duration of AAD once it occurred. The most dramatic impact was in patients with delayed AAD when the duration of disease fell from a median of 18 days in placebo-treated patients to 2.5 days in *S. boulardii*-treated patients. Animal studies have revealed that short term (5 days) treatment with common antibiotics (such as penicillin G, ampicillin, tetracycline, or cefuroxime) had long term effects (2-10 wk) on the normal flora (39-41). In this study, 8/21 cases occurred postantibiotic exposure with incubation periods ranging from 3 to 46 days postantibiotics, indicating that the human colon may also require a significant time period for the normal flora to recover its protective effect. Another effect of *S. boulardii* was to slow the development of AAD, as reflected by the longer incubation periods in the *S. boulardii*-treated group, although the clinical relevance of this finding is unclear. Pharmacokinetic studies have shown that *S. boulardii* is cleared quickly (3-5 days) from the colon after its discontinuation, thus the residual effects of *S. boulardii* found in this study may be due to the normalization of colonic flora facilitated during *S. boulardii* administration and not to a direct action of *S. boulardii* (24-27). In addition, the effectiveness of *S. boulardii* was more pronounced in patients who received multiple antibiotics, which is a group at higher risk for AAD.

The mechanisms of action of *S. boulardii* have been explored by several researchers. Pothoulakis *et al* found that viable *S. boulardii* produces a protease that interferes with *C. difficile* toxin A binding to specific intestinal receptors (42). In addition, *S. boulardii* exerts trophic effects on the intestinal mucosa, resulting in an increase in secretory component, secretory IgA, and in intestinal enzymes such as lactase, maltase, and sucrase (43, 44). Pharmacokinetic studies have also shown elevated stool steady-state levels of *S. boulardii* in antibiotic-treated rat and human volunteers compared with nonantibiotic-exposed controls (26, 27). Thus, in the antibiotic-disturbed intestine at high risk for development of AAD, the yeast is present at high levels ($>10^8$ CFU/g stool).

Other factors that may have influenced the effectiveness of *S. boulardii* were analyzed. The analysis of the relative risks for AAD by specific antibiotic types was limited in this study because of the requirement that all patients be on at least one high-risk ß-lactam antibiotic. The high frequency of study patients receiving multiple antibiotics reflected current clinical prescription patterns, and this clinical practice further increases the risk of AAD to patients. Several studies have shown that the risk of AAD increases not only with ß-lactam antibiotics, but also with the use of multiple antibiotic exposure (1, 16, 22, 28). Other reported risk factors for AAD besides antibiotics have included: age, enteral feeding, recent enemas, use of antacids/H_2 blockers, presence of *C. difficile*, longer lengths of stays in hospitals, and more severe underlying disease conditions (17, 21, 22). *C. difficile* has been reported to be associated with 20-40% of the cases of AAD (1, 3, 19). *C. difficile* was found to be a significant risk factor for AAD in this study (unadjusted relative risk = 2.9) and was cultured in

39% of the patients with AAD. The low number of patients who acquired *C. difficile* limited the multivariate analysis of this factor and also limited the evaluation of the effectiveness of *S. boulardii* in *C. difficile*-positive patients. There was only a 3% power to detect a difference if there was one, thus this may be due to a type II error. In a previous study with more *C. difficile*-positive patients (n = 48), a trend for a lower frequency of *C. difficile*-associated AAD was found in *S. boulardii*-treated patients (3/32, 9.4%) compared with placebo patients (5/16, 31%, $p = 0.07$) (28). In trials that were specifically aimed at the treatment of acute *C. difficile* disease, *S. boulardii* was found to be a significantly effective treatment, especially for patients with recurrent *C. difficile* disease (30, 45, 46). The risk factors found in other studies (length of stay, surgery, enemas, antacid use) were not found to be significant in this patient population.

S. boulardii was shown in this study to be a safe biotherapeutic agent that significantly reduced the incidence of ß-lactam-associated diarrhea, either given alone or with other antibiotics. *S. boulardii* may offer a safe and effective means of preventing AAD in patients in whom diarrhea would be highly undesirable.

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Saccharomyces boulardii for *Clostridium difficile*-Associated Enteropathies in Infants

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Summary: Based on experimental evidence in animals showing that the oral administration of *Saccharomyces boulardii* is effective in reducing morbidity and mortality due to *Clostridium difficile*-induced pseudomembranous colitis, we conducted an open trial to examine the effects of the living yeast, given as primary therapy, in a selected group of infants and children with persistent intestinal symptoms related to toxinogenic *C. difficile* overgrowth. Over a period of 10 consecutive months, we studied 19 eligible patients (median age 8 months) who presented with enteral symptoms lasting for > 15 days and who had solely *C. difficile* in stools with positive cytotoxin B assay. Serotyping of the strains and determination *in vitro* of production of toxins A and B were performed subsequently. The patients presented with persistent or protracted diarrhea, malabsorption, and failure to grow (n = 8), or with repeated attacks of colics, emesis, and hypermeteorism without diarrhea (n = 4), or with both entities (n = 7). Patients with chronic protracted diarrhea (n = 3) had depressed jejunal disaccharidase activities and ultrastructural changes of enterocytes, including sparce and shortened microvilli. None had evidence of colitis. All the strains of *C. difficile* tested (n = 17) belonged to pathogenic serotypes (A_1, A_8, C, F, G, H, and K) and produced *in vitro* high levels of toxins A (n = 16) and B (n = 17). *S. boulardii* was given orally in a lyophilized form over 15 days (250 mg two to four times per day according to age). Within 1 week of treatment, enteral symptoms and physical findings resolved in 18 patients (95%) with marked decreases ($p < 0.001$) in the number of stools, frequency of colic episodes, and total duration of colics per day. Clearing of toxin B was observed within 15 days of therapy in 16 cases (85%), whereas eradication of *C. difficile* from stools was complete after 1 month in 14 (73%). Also, the ultrastructural changes observed in patients with chronic protracted diarrhea (n = 3) had disappeared after 1 month. A clinical and bacteriological relapse occurred in two patients (11%), which resolved rapidly with a second 15-day course of *S. boulardii*. These findings suggest that some toxinogenic strains of *C. difficile* may cause chronic enteropathies without colitis that may be improved by oral administration of *S. boulardii*.

Key Words: *Clostridium difficile* - *Saccharomyces boulardii* - Chronic diarrhea - Infancy.

In adults, *Clostridium difficile* is now recognized as the principal etiological agent of pseudomembranous colitis and of antibiotic-associated diarrhea (1). In neonates and infants, however, its role in intestinal diseases has been questioned (2,3). Asymptomatic carriage of nontoxinogenic as well as toxinogenic isolates is frequent until the age of 2 years (4), and only a few cases of pseudomembranous colitis related to *C. difficile* have been reported in infants (5,6). Although the association of toxinogenic strains of *C. difficile* with chronic diarrhea, Hirschsprung's disease, necrotizing enterocolitis, and relapses of inflammatory bowel disease has been described in children (7-9), systematic antibiotic therapy for children with diarrhea and fecal *C. difficile* toxin remains controversial because of the absence of objective criteria of pathogenicity. The observation that *C. difficile* colonization is mostly the consequence of antibiotic therapy or occurs; commonly in neonates whose intestinal flora is not yet constituted emphasizes the crucial importance of the microflora as a barrier to colonization.

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In humans, experimental rectal infusion of homologous feces as well as the ingestion of mixed bacterial flora have been reported to be efficient in preventing relapses of intestinal symptoms related to *C. difficile* (10,11).

Moreover, the colonization of hamsters with non-toxinogenic *C. difficile* strains before exposure to toxinogenic strains increases survival rate up to 93% as opposed to a 21% survival rate recorded in the control group (12).

Recently, the oral administration of a lyophilized preparation of *Saccharomyces boulardii*, a non-pathogenic yeast, has also been shown to be efficient in preventing mortality due to experimental cecitis in hamsters (13) and to *C. difficile*-induced pseudomembranous colitis in gnotobiotic mice (14). A preliminary study conducted in adults has shown that the oral administration of *S. boulardii* reduced significantly the recurrence rate of postantibiotic *C. difficile* colitis (15). These observations prompted us to conduct an open trial with *S. boulardii* given orally as primary therapy in a selected group of infants and children who presented with persistent intestinal symptoms related to toxinogenic *C. difficile* colonization.

METHODS

Patients

The study was conducted over 10 consecutive months in 19 eligible patients who all were referred to the Cliniques St-Luc, University Hospital, Brussels. Infants and children were excluded from the trial for the following reasons: clinical or endoscopic evidence of pseudomembranous colitis, immune-compromised status, antibiotic therapy for <3 days before enrollment, and discovery in stools of another pathogen. Informed consent was obtained from the parents of all infants, and the study protocol was approved by the University Hospital Ethical Committee. Patients who were monitored for <2 weeks also were considered to have insufficient follow-up and were excluded from the study. Eligible patients had solely positive *C. difficile* stool cultures and also were positive for cytotoxin B assay. Serotyping of the strains and determination *in vitro* of toxin A and B production were performed subsequently. At enrollment, a full medical history was recorded from the parents, including details on diet, stool habits, antibiotic usage, and growth curves. *S. boulardii* (ATCC 74012 Laboratoires Biocodex, Montrouge, France) was given in a lyophilized form (batches of 250 mg) at the following doses: 250 mg two times per day for infants < 1 year of age, three times per day for children 1-4 years of age, and four times per day for those >4 years of age. Administration of the drug was continued for 2 weeks. While the infants were hospitalized, the nursing staff kept a daily record of dietary intakes, episodes of crying, colic attacks, and stool frequency and consistency. For outpatients, the same data were recorded by the parents.

The protocol called for collection of fresh stool samples for *C. difficile* culture on entry into the study and at days 7, 15, and 30. Chronic persistent diarrhea was defined as abnormally frequent (more than three per day) and liquid stools lasting for 14 days or more, and chronic protracted diarrhea as chronic diarrhea lasting for 14 days or more, associated with malabsorption, failure to thrive, and unresponsiveness to conventional therapy. Patients in whom the enteral disease resolved with treatment entered a follow-up period. The parents were asked to report whenever diarrhea or other symptoms recurred. Infants were routinely reviewed at monthly intervals. Microbiologic studies on feces were repeated at day 30 or whenever symptoms recurred. Symptomatic relapse was defined as recurrence of diarrhea and/or enteral symptoms with evidence of *C. difficile* and toxin B in the stools, provided that symptoms had resolved with initial therapy.

Microbiological Methods

Feces were inoculated into the medium described by George et al. (16), which had been modified to include the selective agents cycloserine (350 µg/ml) and cefotaxime (4 µg/ml), and incubated at 37°C for 48 h. *C. difficile* was identified on the basis of fermentation product analysis by gas liquid chromatography. Fecal filtrates were examined for *C. difficile* cytotoxin B using confluent monolayers of HeLa cells with specific neutralization by *C. difficile* antitoxin. The serogroup of each *C. difficile* strain was determined by a slide agglutination technique using 10 rabbit antisera designated by letters A, B, C, D, F, G, H, I, K, and X (17, 18). Strains belonging to serogroup A were further characterized for subclasses (A_1 to A_{12}) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (19). The strains of *C. difficile* isolated from the patients were coded and sent to one of us (G.C.) for *in vitro* analysis of toxin A and B production.

The assays were performed by persons uninformed of the identity of the strains using an enzyme-linked immunosorbent assay for toxin A and a cytotoxicity assay for toxin B (20,21).

Statistical Analysis

Differences between means of the clinical parameters were tested for statistical significance by non-parametric methods (Mann-Whitney U test) (22).

RESULTS

Historical data of the patients are summarized in Table 1. Of the 19 infants and children studied, 13 had received during the months or weeks preceding admission one or more courses of antibiotic therapy for acute infections, including tonsillitis, otitis, bronchitis, enteritis, or malaria. Nine infants had been treated with antibiotics for an acute episode of enteritis related to the presence of a pathogen in their stools.

Disappearance of these pathogens from stools was repeatedly demonstrated before enrollment into the study. The antibiotics incriminated for the induction of *C. difficile* infection were trimethoprim sulfamethoxazole (n = 13), amoxicillin (n = 2), erythromycin (n = 1), cloxacillin (n = 1), colimycin (n = 1), fungizone (n = 1), and chloroquine (n = 1). One patient had received oral vancomycin for protracted diarrhea with weight loss related to the presence of a toxinogenic strain of *C. difficile* in stools. After clinical and bacteriological relapse was confirmed, he was enrolled in the study. The patients presented on admission with persistent or protracted diarrhea (n = 8), with a syndrome of repeated attacks of colic, emesis, and hypermeteorism without diarrhea (n = 4), or with both digestive syndromes (n = 7).

TABLE 1. *Patients and mode of presentation*

Number	19
Boys	7
Girls	12
Age	
Median	8 mo
Range	2 mo to 11 yr
Patients who had received antibiotic therapies before onset	13
Patients who had acute enteritis before onset	9
Pathogens	
Rotavirus	3
Y. enterolilica	3
C. jejuni	1
S. enteritidis	1
C. albicans	1
Mode of presentation of *C. difficile* overgrowth	
Chronic diarrhea	8
Colics with emesis and hypermeteorism	4
Mixed	7

TABLE 2. *Symptoms and clinical findings at enrollment*

General	
Weight loss, failure to thrive	6
Poor appetite	5
Malnutrition	3
Digestive	
Chronic persistent diarrhea	12
Chronic protracted diarrhea	3
Recurrent episodes of colics	11
Hypermeteorism	13
Abdominal distention	8
Repeated emesis	7

Symptoms and physical findings at admission are listed in Table 2. Among the 15 patients who presented with chronic diarrhea, three exhibited clinical and biological evidence of malnutrition with failure to grow and poor appetite. Histological examination of jejunal biopsy samples (n = 3) showed either normal mucosal morphology (n = 2) or partial villous atrophy with increased number of mucoid cells (n = 1). In two patients, the specific activity of jejunal disaccharidases was depressed to values 20-50% of the control values. Electron microscopy of jejunal enterocytes (n = 3) (Fig. 1) showed sparse and shortened microvilli covered by abundant mucoid material containing cellular debris. At rectosigmoidoscopy, however, there was no macroscopic or microscopic evidence of colitis. The patients with chronic protracted diarrhea were placed on supportive parenteral nutrition. Before therapy was initiated, all patients had *C. difficile* with positive cytotoxin B in their stools without any other pathogen detectable. Subsequent bacteriological analysis of 17 isolates from 15 patients confirmed that 16 of the 17 strains tested, produced *in vitro* high levels of both toxins A and B, one strain producing toxin B only (Table 3). Strains belonged to the following serotypes: A_1 (n = 2), A_8 (n = 1), C (n = 1), F (n = 1), G (n = 4), H (n = 5), and K (n = 3). The patients received a mean course of 15 ± 5 days of oral *S. boulardii*.

In 18 patients, symptoms and clinical findings resolved within 1 week of treatment. Compared with initial values recorded by the parents and the nursing staff on day 0, the number of stools, frequency of colic episodes, and total duration of colics per day were decreased significantly ($p < 0.001$) on day 7 (Fig. 2).

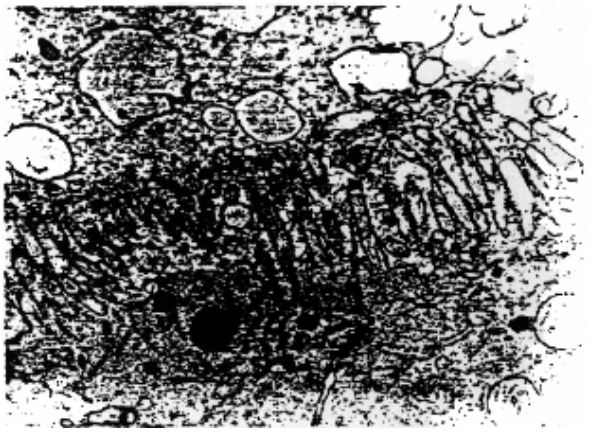

FIG. 1. Electron microscopy of jejunal enterocytes from a 6-month-old girl with protracted diarrhea, malnutrition, and failure to thrive. A strain type H^+ of enterotoxigenic *C. difficile* was discovered in stools. Note shortened and sparce microvilli covered by abundant mucoid material containing cellular debris. Original magnification x29,412; reduced 50% for reproduction.

Clearing of cytotoxin B from stools occurred after 15 days in 16 patients (85%) and after 1 month in two others, whereas eradication of *C. difficile* from stools was complete after 1 month in 14 patients (73%) (Table 4). The ultrastructural changes of jejunal epithelial cells observed in patients with protracted diarrhea, including sparse and shortened microvilli, had disappeared on the control biopsy sample taken 1 month later.

TABLE 3. *Bacteriological data: serotype of C. difficile and in vitro production of toxins A and B*

Patients	Age	Serotype	Toxin A[a] (ng/ml)	Toxin B[a] (pg/ml)
1. D. K	5 mo	A_8	1.2	3.1
2. D.C.	2 mo	H	1.1	3.5
3. B.M.L.	10 mo	H	2.7	2.6
4. K.L.				
1st	4 mo	G	2.3	3.1
2nd	6 mo	G	2.1	3.7
5. L.P.				
1st	11 yr	G	2.3	4.2
2nd	11 yr	G	2.7	3.5
6. A.C.	7 mo	K	2.3	3.6
7. B.T.	32 mo	H	2.6	3.1
8. W.X.	6 mo	H	3.1	3.1
9. N.N.	9 mo	C	0.8	4.5
10. B.S.	6 mo	A_1	2.5	2.6
11. P.S.	3 mo	K	0.5	1.3
12. D.M.	22 mo	K	1.0	2.7
13. D.E.	2 mo	A_1	0.8	1.6
14. C.O.	13 mo	H	0.9	3.1
15. E.M.	4 mo	F	0.0	2.8

[a] Results are expressed as $\log_{10}$.

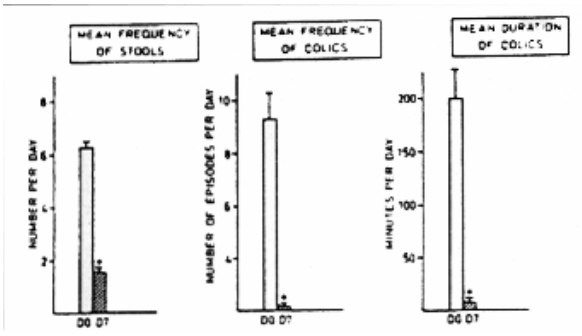

FIG. 2. Frequency of stools, frequency of colics, and duration of colic episodes per day recorded by the parents and nursing staff on day 0 (D0) and after 7 days (D7) of treatment with *S. boulardii*. The number of patients with diarrhea is 15 and those with colic episodes is 11. Values are mean ± SD; *p < 0.001 *versus* data of D0.

Two patients (11%) in whom *C. difficile* had been eradicated were readmitted 2 months later for a clinical and bacteriological relapse consisting of the reappearance of the same symptoms and of the same toxinogenic strain (G) as found at the first episode. A second trial of 2 weeks with oral *S. boulardii* resulted in rapid disappearance of symptoms (7 days) and clearing of *C. difficile* and cytotoxin from stools (15 days). Only one patient failed to respond to *S. boulardii* therapy despite two well-conducted trials of 15 days each. This patient was admitted first at 8 months of age for protracted diarrhea, hypermeteorism, and failure to thrive lasting for 1 month. A toxinogenic strain of *C. difficile* serogroup C was discovered in the stools. After unsuccessful treatment with *S. boulardii*, oral vancomycin (40 mg/kg/day) was initiated, but the drug remained without effect on both symptoms and *C. difficile* excretion. Examination of jejunal and rectal biopsy specimens (conventional histology) showed no abnormality. Enteral symptoms and excretion of the same serogroup C strain with cytotoxin persisted in stools up to the age of 13 months, after which spontaneous disappearance of the strain and catch-up growth were observed.

TABLE 4. *Course and outcome of the patients*

No. of patients	19
Disappearance of symptoms within 1 wk	18
Disappearance of *C. difficile* from stools (total)	14
Within 15 days	9
1 mo	5
Negativation of toxin B (total)	18
Within 15 days	16
1 mo	2
Symptomatic relapses (<2 mo)	2
Reappearance of *C. difficile* and toxin B	2
Response to second trial	2
Failure of response to therapy	1

DISCUSSION

Our study concerns a selected group of infants and children who presented with intestinal symptoms lasting for > 15 days and who all had *C. difficile* and toxin B in feces without other pathogen detectable.

Subsequent *in vitro* analysis on the fecal isolates confirmed that 17 strains tested from 15 patients all produced toxin B, whose 16 strains produced both toxins A and B. Previous studies have shown that *C. difficile* may cause chronic persistent diarrhea in infants and children (7,8). However, up to now, convincing evidence for a pathogenic role of *C. difficile* in infancy was lacking. Many asymptomatic infants colonized with *C. difficile* have detectable toxin B in feces, sometimes at titers equal to or higher than those found in adults with pseudomembranous colitis. Also, prospective studies (2,3,23) with control cases have failed to establish a clear relationship between *C. difficile* colonization, toxin production, and enteral disease.

Up to now, most of these studies have been conducted without benefit of toxin A assay because reagents for toxin A were not commercially available. Although toxin A is obviously as important as toxin B in the pathogenesis of intestinal lesions, asymptomatic neonates may be carriers of toxin A as well as of toxin B, and other pathogenic mechanisms, such as mucosal invasion, have been suggested (24). Evidence indicates that determination of the serotype of *C. difficile* is more reliable for distinguishing between pathogenic and nonpathogenic strains (18,19).

A recent study (7) has shown that some serotypes of *C. difficile* isolated from asymptomatic children, although producing toxin B, were not virulent on an animal model of pseudomembranous coIitis. Using the same approach of serotype analysis, we found that all the strains isolated before therapy was initiated belonged to serotypes commonly found in adults with *C. difficile* colitis and differed from non-virulent serotypes colonizing asymptomatic neonates (4). Pathogenicity of the strains is further attested by the positive concordance between clinical and bacteriological relapses and by the persistence over 5 months in one of the patients of enteral symptoms and of a serogroup C strain, usually detected in adults with severe forms of pseudomembranous colitis. Also, it is noteworthy that three patients presented with protracted diarrhea with ultrastructural changes of enterocytes consistent with a severe enteropathy unrelieved by conventional treatment, which reversed to normal within 1 month after *S. boulardii* therapy. For these patients, the average number of days of diarrhea before entry into the study was 21 days. We have observed similar cases of chronic protracted diarrhea with symptoms lasting for several months to 1 year and cured by the eradication of *C. difficile* from stools. Although the relationship between pathogenic strains of *C. difficile* and enteral lesions remains unclear, the organism has been isolated from the human jejunum and found to adhere to the jejunal mucosa, suggesting that enteral disease due to *C. difficile* has an endogenous origin (25).

The traditional approach of treating patients with *C. difficile* and related intestinal diseases is oral antibiotic therapy. Vancomycin is commonly used in adults with *C. difficile* colitis. However, although initial therapy is usually successful, relapses can occur with rates up to 50% (26,27). Because prolonged treatment with vancomycin does not clear the colon of *C. difficile* spores (27), patients who have one recurrence are even more likely to present with other recurrences. In infants and children, no clear recommendation on its use has been formulated because of the uncertainty of pathogenicity of the isolates and the cost of the therapy. Therefore, vancomycin is usually reserved for patients with confirmed pseudomembranous colitis or severe enteral disease (26). The choice of *S. boulardii* in our study was based on experimental data showing that mortality in hamsters due to clindamycin-induced cecitis was markedly decreased when the animals received the yeast (13) and on clinical evidence showing a success of this therapy in 85% of adults who had multiple recurrences of colitis after vancomycin therapy (15).

A recent prospective trial (28) has clearly demonstrated the efficacy of oral *S. boulardii* over a placebo in reducing postantibiotic-associated diarrhea in a selected group of adults at high risk for pseudomembranous colitis. Our findings in infants indicate that oral administration of the yeast resulted in prompt disappearance of symptoms (95%), with negativation of toxin B and eradication of *C. difficile* within 15 days in respectively 85% and 73% of the patients. Negativation of toxin B in stools reached 95% after 1 month. Patients with clinical and bacteriological relapses responded rapidly to a second course of *S. boulardii* with no more relapse thereafter.

The mechanism by which *S. boulardii* protects against *C. difficile* colonization and toxin production is unknown, although several properties of the yeast may contribute to explain its efficacy. In animal models of *C. difficile* overgrowth, it has been shown that the oral administration of *S. boulardii* increases intestinal resistance of the host to *C. difficile* colonization and decreases the levels of toxin B production (14-29). In a cell culture model, *S. boulardii* can prevent cytotoxicity of toxins A and B by a protective effect of the yeast on cell cytoskeleton (30). In addition, the oral administration of *S. boulardii* enhances disaccharidase activity in the intestinal mucosa of humans and rats (31), as well as the intestinal production of secretory component and s-IgA (32). Although the efficacy of *S. boulardii* as primary therapy for all forms of *C. difficile* colonization awaits definitive confirmation by placebo-controlled trials, the present study suggests that this yeast may be an effective therapy in patients with persistent enteropathies related to *C. difficile*.

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ACTUALITES THERAPEUTIQUES

AIDS-RELATED DIARRHEA: a double-blind trial of *Saccharomyces boulardii*

T. SAINT-MARC [1], H. BLEHAUT [2], C. MUSIAL [2], J.L. TOURAINE [1]

SUMMARY:

Chronic AIDS-related diarrhea remains a problem because of its persistence and variable response to available treatments. *Saccharomyces boulardii* (Sb) is a nonpathogenic yeast used to treat diarrhea with gut microflora alterations. This double-blind, placebo-controlled, parallel-group trial in 35 patients with Stage IV AIDS was conducted to evaluate the efficacy of Sb in AIDS-related diarrhea unresponsive to standard therapy. Mean age was 34.9 years. Most patients were male. The cause of the diarrhea was identified in 54.3% of cases (cryptosporidiosis in 20%). Eighteen patients were assigned to Sb therapy and 17 to placebo therapy. The two groups were comparable at baseline. Resolution of diarrhea was recorded in 61% of Sb patients *versus* 12% of placebo patients after one week ($p < 0.002$). Significant improvements were also noted in the Sb group regarding the daily diarrhea score based on stool number, weight, and volume ($p < 0.002$); abdominal pain; abdominal distension; asthenia; weight gain; and the Karnofsky index. Tolerability was outstanding. Our data show that over a one-week period Sb is an effective symptomatic treatment for persistent AIDS-related diarrhea. The pathophysiologic mechanism under-lying this beneficial effect and the long-term efficacy of Sb remain to be determined. SB was as effective in relieving diarrhea in this study as in previously published studies in other indications.

KEY - WORDS: Acquired Immuno-deficiency Syndrome. - Diarrhea. - Severity of illness index. - *Saccharomyces boulardii.*

INTRODUCTION

Gastrointestinal problems are very common in AIDS; they are seen in 50 to 90% of cases, most often in the form of chronic diarrhea. Such diarrhea generally responds poorly to treatment and varies greatly in severity from mild to very severe (15 liters/day) forms with life-threatening dehydration and malnutrition (1, 2, 3).

The etiology and pathophysiology of such diarrhea are complex and multifactorial: immune deficiency, villous atrophy with malabsorption, inflammation of the mucosa, massive and repeated antibiotic treatment, and bacterial (*Mycobacterium avium, Campylobacter, Clostridium difficile*, etc.), fungal (*Candida*), viral (*Cytomegalovirus - CMV, Adenovirus, Herpes virus)* or parasitic (*Cryptosporidium, Microsporidium, Giardia lamblia*, etc.) infections. The etiology of diarrhea is often impossible to determine and might then be explained by direct attack of enterocytes by HIV (4).

The action of etiologically-based treatments (anti-infectious, anti-parasitic) in AIDS-related diarrhea is irregular. That of symptomatic treatments (inhibitors of intestinal function, regulators of water secretion) also varies and they are not always free of adverse effects. Octreotide (5) and acetorphan (6) have been evaluated in controlled trials in limited groups of patients.

Saccharomyces boulardii (Sb) is a non-pathogenic yeast used in many countries in the treatment of non-specific diarrhea and imbalance of the gut flora. Its activity has been extensively studied in various open or double-blind trials: gastroenteritis in children, antibiotic-associated diarrhea (7, 8), *Clostridium difficile*-associated diarrhea in adults (9) or children (10) and diarrhea related to enteral alimentation via tube (11, 12). In addition, tolerability of this yeast is excellent, even in very fragile patients, such as in resuscitation (11) or in major burns patients (12) and no serious adverse reactions have been reported since it first came on to the market in 1962.

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Study sponsored by Biocodex Laboratories.

Several mechanisms could explain the action of Sb in diarrhea: fungal antagonism, in particular against *Candida*, studied in the animal (13); diminution of the pathogenic effects of bacterial toxins, extensively studied with *Clostridium difficile* toxin and cholera toxin (14) and which appears to be dependent upon substances produced by Sb in contact with the intestinal mucosa (15, 16); stimulation of intestinal immune defences (17), increased intestinal disaccharidase activity (18).

An initial open clinical trial of Sb in AIDS-related diarrhea yielded very positive results (19). Seventeen patients with chronic diarrhea were treated with 3 grams of product per day for 2 weeks. The daily number of stools decreased from 9.0±3.2 before treatment to 2.1±0.9 after and there was marked weight gain.

There were no adverse effects of Sb and it is probable that its tolerability in this context is excellent, despite a very slight potential risk of blood penetration of Sb in immunodeficient individuals. In actual fact, this risk is theoretical and administration of very high doses of the product to nude mice has provided no evidence of any dissemination of the yeast (20).

It was thus appropriate to evaluate the symptomatic activity and tolerability of Sb in AIDS-related diarrhea in a randomized, double-blind, placebo-controlled trial.

METHODS

Eligibility criteria

Volunteers having given their informed consent in writing, with AIDS, aged 18 years or over, on an oral diet, with diarrhea (3 liquid stools or more per day), regardless of the cause, unresponsive to standard etiological or symptomatic treatment, were eligible. The protocol was approved by the Institutional Review Board of Edouard Herriot Hospital.

Patients on antifungal treatment by enteral administration, or parenterally with elimination via the gut, or symptomatic treatment for diarrhea were not eligible, the same applying to unreliable patients (drug addicts, etc.).

Assessment of patients

Patients, hospitalized or under care of the out-patient clinic of the hospital's AIDS unit, underwent initial evaluation enabling the confirmation of inclusion criteria, and recording of the history of their illness and of treatment taken. Their general status was assessed using the Karnofsky index (21). History and physical examination covered general symptoms, gastrointestinal symptoms, history of diarrhea, etiologic work-up and study of its characteristics. Investigations included complete blood count, electrolytes, protein electrophoresis, ASAT, ALAT, creatinine, stool culture and fecal parasitology. Colonoscopy was performed according to symptoms. Patients were then randomly assigned and treatment given for 7 days before a further clinical and laboratory evaluation.

Precise monitoring of disturbed intestinal function was based upon the Daily Diarrhea Score or D.D.S (Table I) developed by G. Hart (22) for assessment of the severity of diarrhea related to enteral alimentation. This simultaneously takes into account the number of stools, their consistency and their approximate volume. Despite its apparently subjective nature, this assessment of volume is highly reproducible in a given individual, enabling good quality monitoring.

TABLE I. - Daily diarrhea score values

Criteria	Small volume	Normal volume	Large volume
Consistency			
- normal	1	2	3
- soft	3	6	9
- liquid	5	10	15

All trial drop-outs were to be analyzed for tolerability and, if possible, for efficacy.

Treatment

In this double-blind, two-parallel groups trial, each randomized patient immediately started placebo or active treatment according to advance assignment using the weighted coin method. This technique provided well balanced groups without the disadvantages of block assignment. Sb, the active product, was given as 3 daily divided doses, each of two 500 mg sachets. The dose was the same for all patients. The placebo was identical in terms of appearance and general characteristics. Administration was by the oral route, the product being mixed with food just at the time or administration, or via gastric tube. If a dose was omitted, it had to be given together with the following dose. Omission of a whole day of doses led to premature termination of the trial for the patient concerned for inadequate compliance. Reasons for all trial drop-outs were to be documented. All side-effects were noted and an assessment made of causal relation. If the patient requested, treatment could be continued beyond 7 days on an open basis with careful clinical supervision.

Apart from antifungal and symptomatic anti-diarrhea agents, all other necessary treatment was permitted and noted in the case report form. Whenever possible, diet was to remain the same throughout the trial.

Statistics

It was decided to enroll 40 cases in 2 parallel groups for this first double-blind trial. All enrolled patients were analyzed. Findings were noted in a case report form then coded anonymously and processed using PCSM (Version 5.0, Deltasoft, Grenoble, France) software. Mann-Whitney's non-parametric U test was used for quantitative variables. Tests used for qualitative variables were the Chi^2 test or Fisher's exact probability calculation when calculated numbers were too small (less than 5 in 2 x 2 tables). Adverse events were to be analyzed case by case.

RESULTS

Results are expressed as means (± standard deviation) for quantitative data and as the number of cases and percentage for qualitative data.

At the end of the 18 months of the study, none of the 36 assigned patients had been lost to follow-up. However, one could not be analyzed since his results were not available on D7. This 31-year-old patient had cerebral toxoplasmosis and pulmonary and renal tuberculosis, associated with diarrhea for 3 weeks. The day after treatment was started, his condition deteriorated with the onset of coma preventing oral administration of the product. He had been assigned to the placebo group. This case was not taken into account in the analysis below, this worsening being clearly due to cerebral toxoplasmosis and not the diarrhea or its treatment.

Of the 35 evaluable cases, 18 were in the Sb active product group and 17 in the placebo group.

TABLE II. - Study population: main characteristics.

	S. boulardii **N = 18**	**Placebo N = 17**
Gender		
- male	17 (94.4%)	16 (94.1%)
- female	1 (5.6%)	1 (5.9%)
Age (yrs)	33.9 ± 8.6	35.9 ± 9.1
Weight (kg)	58.1 ± 11.6	59.3 ± 9.7
Probable etiology of diarrhea		
- cryptosporidiosis	3 (16.7%)	4 (23.5%)
- cytomegalovirus	4 (22.2%)	0 (0.0%)
- mycobacteria	3 (16.7%)	2 (11.8%)
- miscellaneous	1 (5.6%)	2 (11.8%)
- no etiology found	7 (38.9%)	9 (52.9%)
Number of stools	4.9 ± 2.1	4.5 ± 1.7
Daily Diarrhea Score (D.D.S.)	58.9 ± 33.4	51.9 ± 25.9
Karnofsky index	73.9 ± 17.2	73.2 ± 11.3

Study groups, retrospective analysis

Distribution of principal prognostic factors was very similar between the Sb and placebo groups, enabling valid comparison after treatment (Table II). All patients had full-blown AIDS (CDC (Centers for Disease Control) Stage IV). Gender ratio imbalance was due to the inclusion of a very large majority of homosexual patients, the latter being generally more cooperative than drug addicts. Etiology of diarrhea varied and was often difficult to attribute to any single diagnosis, certain patients having several possible reasons for their intestinal problems. Diarrhea with a definite diagnosis included cases of CMV gut involvement confirmed by endoscopy, and cryptosporidiosis. No etiology was found in 46% of cases. No evidence was found of any statistically significant difference between the two groups concerning any of the above parameters, the same applying to general symptomatology (central temperature, asthenia), concomitant gastrointestinal manifestations (appetite, abdominal pain, flatulence, nausea, vomiting, all graded from 0 to +++), and main combined treatment (anti-toxoplasmosis, various antivirals, anti-pneumocystosis, anti-mitotics, etc.). All patients were on anti-retrovirus treatment (AZT). Similarly, there were no differences concerning main laboratory parameters (CBC including T4 and T8 count, electrolytes, baseline liver or renal function tests, etc.). Only plasma calcium showed a difference between the two groups (2.14±0.12 mEq in the Sb group and 1.99±0.29 in the placebo group). This difference concerning a factor of no theoretical prognostic importance could be attributable to simple sampling fluctuations related to the large number of comparisons made in this retrospective analysis.

Table III. - Treatment results on D7.

	S. boulardii **N = 18**	**Placebo N = 17**
Number of stools [2]	2.0 ± 1.5	4.4 ± 1.6
Daily Diarrhea Score (D.D.S.) [2]	15.5 ± 21.8	52.9 ± 28.5
Variation in D.D.S.* (DO-D6) [2]	-43.4 ± 21.4	+0.9 ± 16.5
Variation in Karnofsky index [1]	+5.6 ± 7.0	-1.8 ± 3.9
Variation in weight (kg) [2]	+2.0 ± 1.8	-3.1 ± 2.2

(1) $p<0.05$; (2) $p<0.002$
* D.D.S. = Daily Diarrhea Score

Disturbed intestinal function at end of trial (D7)

Results of the two treatments compared are shown in Table III.

Absence of diarrhea, defined by Hart as a D.D.S below 13, was found on D7 in 61% of cases (11/18) treated with Sb as against 12% of those (2/17) receiving the placebo ($p<0.002$). Improvement in D.D.S. in the Sb group was gradual but visible starting from the second day of treatment and persisted until the end of the evaluation period. In the placebo group, the score remained very stable during the same period. Results concerning the number of stools per day followed exactly the same pattern but the difference was quantitatively less marked because of the amplification effect introduced by the D.D.S. in the assessment of diarrhea.

Other evaluation criteria on D7

While results concerning weight (59.8±12.6 kg in the treated group vs. 57.6±9.2 kg in the placebo group) and the Karnofsky index (79.4±11.6 in the treated group vs. 71.5±11.4 in the placebo group) were less striking than those regarding diarrhea, improvements during treatment with Sb were still statistically significant.
Other symptoms and signs also improved with Sb, sometimes reaching statistical significance. The following were evaluated: temperature (N.S.), asthenia ($p<0.05$), anorexia (N.S.), abdominal pain ($p<0.05$), flatulence ($p<0.01$) and the existence of nausea or vomiting (N.S.). There was thus a clear improvement in those manifestations directly related to diarrhea symptomatology.

Tolerablity

No adverse events nor any particular signs of infection were seen in this trial. The only complaint expressed by patients concerned the taste of treatment, which was found to be particularly unattractive by those with anorexia and taking a large number of medicines. Among the 16 sets of laboratory studies obtained before and after treatment, plasma potassium and hemoglobin showed a slight but significant increase in the group treated with Sb. No patient dropped out of the trial.

Subsequent follow-up

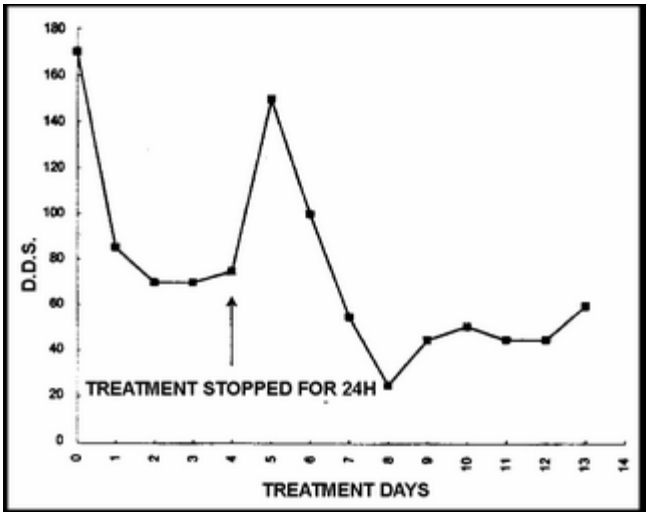
The majority of patients on Sb wished to continue treatment on an open basis after the trial. All placebo patients with a treatment failure were also offered Sb on an open basis if they wished. No clinical or paraclinical adverse events have occurred in the patients followed up for a longer time. As would be expected from the natural history of AIDS, all patients died within a month to a year after the end of this trial.

DISCUSSION

This retrospective trial was designed to assess the action of Sb on AIDS-related diarrhea. Results showed the clear efficacy of the product regarding diarrhea, whether in terms of the existence or not of diarrhea, the number of stools per 24 hours or Daily Diarrhea Score (D.D.S.). Subtracting the placebo effect of approximately 12%, it can be considered that diarrhea disappeared in one patient out of two under the influence of Sb treatment. Almost all patients derived benefit in terms of variations in their D.D.S (Fig. 1). This improvement in intestinal function was accompanied by a useful effect on body weight and quality of life measured by the Karnofsky index. This improvement was slight with regard to D7 raw data but statistically significant in terms of D0-D7 variations in these criteria. Weight gain of 2 kg in a week was marked, related much more to rehydration, becoming effective when diarrhea stopped, than to actual corrected nutritional status. The general condition of the patients improved (less asthenia) as did their gastrointestinal symptoms. Treatment predictably had no influence on vomiting.

Case No 2 offers a typical illustration of the action of Sb. This 28-year-old woman had severe cryptosporidiosis with more than 10 stools per day. There was a rapid improvement in her D.D.S (Figure 1), though without disappearance of diarrhea. Failure to comply with treatment for one day resulted in an immediate recurrence of symptoms, followed in turn by a marked improvement when treatment was restarted and subsequently continued until the 14th day.

Fig.1. - Case 2. Daily diarrhea score.



While the tolerability of Sb was excellent in all patients, with a total absence of adverse effects, acceptability of the product was mediocre in both groups. The taste of the product was considered unpleasant by 57% of patients who were not only anorexic but also required to ingest many other medicines each day. The pharmaceutical form used contained no sweetener and even the placebo was flavored with a yeast aroma in order to obtain maximum resemblance with the active product.

In short, this trial confirmed the efficacy of Sb on the symptom of AIDS-related diarrhea, confirmed by accompanying variations in Karnofsky index, body weight and a slightly but significantly higher plasma potassium in the Sb group after treatment.

Any precise pathophysiologic interpretation of these findings is difficult in view of the complexity of factors involved in AIDS-related diarrhea. However, it is reasonable to propose several hypotheses:

- Sb has been experimentally to enhance gut immunity (17) but this mechanism of action appears hypothetical in view of the very rapid onset of therapeutic effect.
- A trophic action of Sb on the intestinal mucosa has already been shown in the healthy subject, with in particular an increase in intestinal disaccharidases (lactase, sucrase and maltase) (18). AIDS patients are known to have decreased intestinal disaccharidase levels (23). This explanation is potentially open to discussion.
- Almost all patients were taking antibiotics and it is quite possible, in the absence of a precise etiology, that these cases of diarrhea were in part antibiotic-related. It was unfortunately impossible, for technical reasons, to test for the presence of *Clostridium difficile* in feces. The efficacy of Sb in this disorder is extensively documented, both clinically (7, 8, 9, 10) and pharmacologically (15, 24).

The complexity, polymorphous pathophysiology and rapidly advancing understanding of AIDS-related diarrhea are such that it is difficult to seek a single mode of action in an explicative pharmacological study aimed at interpreting events seen.

A similar degree of anti-diarrhea effect has already been reported double-blind with Sb in several studies devoted to the prevention of diarrhea: 4.5% diarrhea with Sb as against 17.5% with placebo found by Adamet et al. (7) in the prevention of antibiotic-associated diarrhea, 9.5% as against 21.8% according to Surawicz et al. (8) in the same diagnostic indication, 2.0% as against 13.3% reported by Schlotterer et al. (12) in the prevention of diarrhea during continuous flow enteral alimentation, and 8.7% as against 16.9% found by Tempe et al. (11) in the same context. In our own therapeutic trial, results were 38.9% residual diarrhea with Sb as against 88.2% with placebo.

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