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Evaluation of an Oral Suspension of VP20621, Spores of Nontoxigenic *Clostridium difficile* Strain M3, in Healthy Subjects

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VP20621, spores of nontoxigenic *Clostridium difficile* (NTCD) strain M3, is protective against challenge with toxigenic strains in hamsters. Human administration and colonization may prevent primary *C. difficile* infection (CDI) or recurrent CDI. Healthy adult subjects 18 to 45 years old or ≥60 years old received single or multiple doses of an oral suspension of VP20621 (10^4, 10^6, or 10^8 spores) or placebo. Group 4 (≥60 years old) received oral vancomycin for 5 days, followed by 14 days of VP20621 or placebo. Subjects were monitored for safety and followed through day 28. Stool was cultured for *C. difficile* before, during, and after VP20621 administration. Isolates were tested for toxin by enzyme immunoassay, and VP20621 was confirmed by molecular typing. After single escalating doses, no subjects had *C. difficile*-positive stool cultures. VP20621 was found in the stool of all subjects given 10^8 spores twice a day. Following vancomycin administration, VP20621 was detected in the stool of all subjects given 10^4, 10^6, or 10^8 spores daily beginning on day 2 to 6. Recovered isolates were toxin negative and confirmed to be VP20621. There were no serious adverse events, and no subjects prematurely discontinued study drugs. Following vancomycin administration, 2 placebo subjects became colonized with toxigenic *C. difficile* and 3 placebo subjects became colonized with VP20621. Persistent colonization with VP20621 was detected in stools on days 21 to 28 in 44% of subjects. VP20621 was well tolerated and able to colonize the gastrointestinal tracts of subjects pretreated with vancomycin. Further study of VP20621 to prevent CDI in patients is warranted.

Over 25 years ago, studies in hamsters and patients suggested that colonization with nontoxigenic *Clostridium difficile* (NTCD) could at least transiently prevent *C. difficile* infection (CDI) caused by toxigenic strains (3, 11, 15). These studies include the only prior experience with human administration of NTCD to two patients with recurrent CDI, both of whom showed improvement (11). Over a decade later, additional studies demonstrating the colonization protection of NTCD in hamsters were published, and this time, evidence of not only transient protection but also long-term protection against challenge with toxigenic strains of *C. difficile* was shown (8, 10). The NTCD strains used in the later studies were obtained from hospitalized patients, identified using restriction endonuclease analysis (REA) typing, and selected for their high frequency of isolation from colonized patients (2, 10).

Observational studies in hospitalized patients demonstrated that patients asymptomatically colonized with *C. difficile* (either toxigenic or NTCD strains) were at significantly reduced risk of CDI compared to patients in the same ward environments who were not colonized (12). Colonization with NTCD was documented in 88 of 192 (46%) colonized asymptomatic patients, suggesting that acquisition of NTCD in the hospital setting occurs frequently, presumably as a result of ingestion of spores found in the hospital environment or on the hands of health care workers. Furthermore, 92% of colonized patients had documented prior antimicrobial exposure, suggesting that this is an important prerequisite to colonization. Although the mechanism by which colonization prevents CDI is not known, it is postulated that at least some NTCD strains are capable of using the same colonization niche used by toxigenic strains to impair their colonization. Patients who are colonized with toxigenic strains of *C. difficile* and remain symptom free are presumed to have antibodies directed at *C. difficile* toxins that prevent CDI symptoms but do not prevent colonization (6, 7).

These observations suggest that deliberate administration of NTCD to patients at high risk of CDI, both those receiving antimicrobials in a health care environment and those who have been treated for CDI with an antimicrobial, could reduce the incidence of both primary and recurrent CDI. In this paper, we describe the outcome of administration of suspensions of specific NTCD spores of REA type M3, designated VP20621, to adult subjects to assess both the safety and colonization effectiveness of single and multiple doses in both younger and older subjects.

(This study was previously presented in part in abstract form at the 10th Biennial Congress of the Anaerobe Society of the Americas, Philadelphia, PA, 7 to 10 July 2010 [13] and at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, MA, 12 to 15 September 2010 [14].)

MATERIALS AND METHODS

Study design. This randomized, double-blind, placebo-controlled study was performed at one study center in Basel, Switzerland, from August 2009 to April 2010. The study was approved by the Ethics Committee of the Two Basels (ECTB) in Switzerland. VP20621 doses of 10^4, 10^6, and 10^8 spores were evaluated in 5 study groups (Table 1). Within groups 1, 2, and 4, separate cohorts were enrolled sequentially to evaluate escalating doses of VP20621, with successive cohorts enrolled only after establishing the safety of the dose in the prior cohort. On the basis of observed safety

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TABLE 1 Description of study groups[a]

Group (randomization)	Age range (yr)	Pretreatment	No. of subjects/ dose level	Dose	Regimen
0 (1 active/1 placebo)	18–45	None	1	10^4	Single dose
			1	Placebo	Single dose
1 (4 active/1 placebo per dose cohort)	18–45	None	4	10^4	Single dose
			4	10^6	Single dose
			4	10^8	Single dose
			3	Placebo	Single dose
2 (4 active/1 placebo per dose cohort)	≥60	None	4	10^4	Single dose
			4	10^6	Single dose
			4	10^8	Single dose
			3	Placebo	Single dose
3[b] (8 active/4 placebo)	≥60	None	8	10^8	BID for 5 days, 10 doses total
			4	Placebo	BID for 5 days, 10 doses total
4 (9 active/3 placebo per dose cohort)	≥60	Vancomycin (125 mg QID for 5 days)	9	10^4	QD for 14 days, 14 doses total
			9	10^6	QD for 14 days, 14 doses total
			9	10^8	QD for 14 days, 14 doses total
			9	Placebo	QD for 14 days, 14 doses total

[a] Abbreviations: BID, twice daily; QD, once daily; QID, four times daily.
[b] Per protocol, subjects in group 3 were to receive the highest tolerated dose administered in group 2, which was 10^8.

results, dose escalation proceeded as planned and without study interruption.

Subjects and eligibility criteria. Subjects were healthy volunteers without a known gastrointestinal (GI) disease or disorder affecting the regular function of the lower GI tract who gave written informed consent to participate. Subjects in groups 0 and 1 were ages 18 to 45 years and were not taking any prescription or nonprescription drugs except for estrogen/progestin products for birth control or acetaminophen, as needed. Those in groups 2, 3, and 4 were ≥60 years old and could continue any stable doses of prescription medications, but new prescription or nonprescription drugs (except for acetaminophen, as needed) were not permitted. Subjects were excluded if they had taken antibiotics within the prior 3 months, had a known immunodeficiency disease or hepatitis B or C virus infection, or were pregnant or breast-feeding. Group 4 subjects also were excluded if they had known intolerance to vancomycin.

During screening, all subjects recorded all bowel movements in a diary for 1 week prior to study enrollment. Eligible subjects had to record at least 4 bowel movements during this total time period but were excluded if ≥4 bowel movements on any 1 day were recorded.

Study procedures and dosing. In groups 0 to 3, all subjects were confined to the study unit from the day before dosing (day −1) through day 7. In group 4, subjects were confined to the study unit from day −6 through day 14. Subjects in group 4 received 5 days of pretreatment with open-label oral vancomycin (Vancocin HCl, 125-mg capsules; ViroPharma Incorporated) prior to receiving study drug, with the intention of disrupting the natural intestinal microbiota to simulate the clinical situation in which most *C. difficile* infections occur. All subjects were randomized prior to dosing on day 1.

Study drug was administered as an oral liquid, which was supplied in unit-dose vials. Each vial contained 10^4, 10^6, or 10^8 VP20621 spores in 10 ml of 0.02% polysorbate 80 in water; placebo vials were identical in appearance but contained no spores. Prior to dosing, an additional 30 ml of water was added to each vial, for a total volume of 40 ml per dose. The dose of study drug was administered in the morning prior to breakfast. In group 3 (twice-daily dosing for 5 days), the second daily dose was administered 12 h after the first dose, irrespective of mealtimes.

Safety was monitored through the recording of adverse events (AEs) and changes in physical examinations, vital signs, 12-lead electrocardiogram (ECG), and clinical safety laboratory testing.

While in the study unit, all subjects provided daily stool or rectal swab samples for *C. difficile* culture. Subjects returned to the study unit for follow-up visits and *C. difficile* stool culture on days 14 and 28 in groups 0 to 3 and on days 21 and 28 in group 4. Subjects who had a positive *C. difficile* stool culture at day 28 were asked to return for follow-up (as part of a separate protocol) every 1 to 3 months to assess lower GI adverse events, serious adverse events, and *C. difficile* stool culture (until *C. difficile* stool culture was negative).

Microbiology. Refrigerated stool ($n = 872$) or rectal swab ($n = 7$) cultures were performed at Viollier AG (Basel, Switzerland) under anaerobic conditions on CLO-selective medium (bioMérieux, Marcy l'Etoile, France) and incubated for 48 h. Selected isolates from *C. difficile*-positive cultures were tested for the presence of *C. difficile* toxins A and B using an enzyme immunoassay (*C. difficile* toxin A/B II; TechLab, Inc., Blacksburg, VA). Selected isolates were analyzed by pulsed-field gel electrophoresis using restriction enzymes MluI and XhoI (Molecular Epidemiology, Inc., Lake Forest, WA). Restriction fragment length polymorphism patterns were compared to those for control samples to confirm the identity of the VP20621 strain.

For group 4 only, all day 21 and 28 stool samples were also cultured by the Microbiology Reference Laboratory (Hines VA Hospital, Hines, IL) using cycloserine-cefoxitin-fructose-agar containing taurocholate (TCCFA) medium. *C. difficile* isolates cultured in the Hines laboratory (as well as selected *C. difficile* strains isolated at Viollier) were molecularly typed using REA (2).

Analyses. The sample sizes for the various groups in this first-in-human phase 1 study were those considered adequate for initial assessments of safety and tolerability. No statistical testing was performed. Results are presented using descriptive summaries for the study population, classified by age group and dose level. Data for subjects in the single-dose groups (groups 0 to 2) are combined and presented by treatment group (placebo or VP20621) because of the small numbers of subjects. Adverse events were defined as events that started or worsened during administration of study drug or within 7 days after the last dose of study drug.

RESULTS

Subject characteristics. Between August 2009 and April 2010 a total of 80 subjects were randomized and treated with study drug (60 received VP20621, 20 received placebo), and all completed the study. Subject demographics are shown in Table 2. In both of the multiple-dose groups (groups 3 and 4), subjects ranged in age from 60 to 73 years.

***C. difficile* testing. (i) Groups 0 to 2.** In the single-dose groups, stool samples collected on days −1, 7, and 28 were cultured for the

TABLE 2 Study population demographics

Demographic characteristic	Groups 0 to 2[a]		Group 3		Group 4[b]	
	Placebo ($n = 7$)	VP20621 ($n = 25$)	Placebo ($n = 4$)	VP20621 ($n = 8$)	Placebo ($n = 9$)	VP20621 ($n = 27$)
Age (yr)						
Mean (SD)	51 (15.6)	49 (16.5)	70 (4.4)	64 (3.4)	64 (3.7)	64 (3.7)
Median (range)	44 (29–70)	43 (20–72)	71 (64–73)	64 (60–70)	64 (61–73)	64 (60–73)
No. (%) of subjects						
Male	6 (86)	20 (80)	3 (75)	5 (63)	4 (44)	19 (70)
Caucasian	7 (100)	25 (100)	4 (100)	8 (100)	9 (100)	27 (100)

[a] For groups 0 to 2, data are combined across all single-dose groups/cohorts.
[b] For group 4, data are combined across dose cohorts.

presence of *C. difficile*. No subjects had *C. difficile* detected in stool culture.

(ii) Group 3. Stool samples collected predose (day −1) were negative for *C. difficile* in all subjects. All 8 VP20621-treated subjects had *C. difficile* detected in stool cultures at multiple time points during study drug administration (Table 3), with the first positive culture detected between days 2 and 4. In the VP20621 group, *C. difficile* was detected in 6/8 subjects on day 7 (2 days after the end of dosing), but no subjects had *C. difficile* detected on day 14 or 28. All *C. difficile* isolates detected in group 3 tested negative for *C. difficile* toxins A and B and are presumed to represent the VP20621 strain, but genotypic testing was not performed. No placebo subjects had *C. difficile* detected in stool.

(iii) Group 4. Stool samples collected prior to vancomycin pretreatment (day −6) were negative for *C. difficile* in all subjects. Culture results during daily study drug administration (days 2 to 14) and follow-up (days 21 and 28) are shown in Tables 4 to 6. For each VP20621 dose, *C. difficile* culture results were positive for 9/9 subjects at multiple time points during the 14-day dosing period (with the first positive culture detected between days 2 and 6). After dosing (days 21 or 28), 4/9 subjects at each VP20621 dose had positive cultures. *C. difficile* toxin testing was performed on the first and last positive cultures from all VP20621 subjects, and all were negative for *C. difficile* toxins A and B. Genotypic testing of selected toxin-negative isolates confirmed these to be *C. difficile* strain VP20621 (shaded cells in Tables 4 to 6).

Among subjects who received placebo, 1 subject in each of the first two cohorts had positive *C. difficile* cultures during and/or after dosing. The isolates from these 2 placebo subjects were *C. difficile* toxin A/B positive, but the subjects had no GI symptoms. In the third cohort, 3/3 placebo subjects had positive *C. difficile* cultures during dosing and 1/3 had a positive culture after dosing. The first and last positive cultures of samples from these placebo subjects tested negative for *C. difficile* toxins A and B, and all typed as VP20621.

Five of 6 subjects in group 4 with positive *C. difficile* cultures on day 28 consented to poststudy follow-up: subjects 14102 and 14105 (10^4 dose), 14205 (10^6 dose), 14305 (10^8 dose), and 14310 (treated with placebo). At 1 month follow-up, only 2 subjects (14102 and 14305) remained NTCD positive, and these 2 subjects were negative for NTCD at 3 months.

Safety. VP20621 was well tolerated in all study groups and dose cohorts. No subjects interrupted or were discontinued from study drug due to an AE, and there were no serious AEs. Results of clinical laboratory evaluations, ECGs, and vital signs measurements were unremarkable and did not suggest any abnormalities related to VP20621.

(i) Groups 0 to 2. In the single-dose groups, AEs were reported by 1/7 (14%) placebo subjects and 4/25 (16%) VP20621-treated subjects. GI events were reported by 2 VP20621-treated subjects: 1 subject (10^4 dose) with mild abdominal pain and another subject (10^6 dose) with a "streak of blood in stool." There were no reports of diarrhea or loose/watery stools in groups 0 to 2.

(ii) Group 3. AEs were reported by 0/4 placebo subjects and 5/8 (63%) VP20621 subjects. GI events were reported by 3 VP20621 subjects: 2 subjects with a mild burning sensation on the tongue and another subject with mild flatulence and mild nausea. There were no reports of diarrhea or loose/watery stools in group 3.

(iii) Group 4. A total of 43 subjects were pretreated with oral vancomycin for 5 days, and of these, 36 were randomized and treated with VP20621 or placebo. During the vancomycin pretreatment period, 12/43 (28%) subjects had AEs, including 8/43 (19%) subjects with 1 or more GI AEs. Subjects with these GI events included 3 subjects with mild diarrhea or loose/watery stools; 2 subjects with mild abdominal pain/upper abdominal pain; and 1 subject each with mild abdominal distension, mild

TABLE 3 *C. difficile* stool culture results for group 3

Group and subject no.	Stool culture result[a]							
	Predose (day −1)	Study drug administration days				Follow-up days		
		2	3	4	5	7	14	28
Placebo								
10302	−	−	−	−	−	−	−	−
10304	−	−	−	−	−	−	−	−
10308	−	−	−	−	−	−	−	−
10311	−	−	−	−	−	−	−	−
VP20621 (10^8 spores)								
10301	−	−	−	+ (n)	+ (n)	+ (n)	−	−
10303	−	+ (n)	+ (n)	−	−	−	−	−
10305	−	−	+ (n)	+ (n)	−	−	−	−
10306	−	−	+ (n)	+ (n)	−	+ (n)	−	−
10307	−	+ (n)	−	+ (n)	−	+ (n)	−	−
10309	−	−	+ (n)	+ (n)	+ (n)	+ (n)	−	−
10310	−	+ (n)	+ (n)	+ (n)	+ (n)	+ (n)	−	−
10312	−	−	+ (n)	−	+ (n)	+ (n)	−	−

[a] Symbols and abbreviations: −, *C. difficile* culture negative; +, *C. difficile* culture positive; (n), toxin A/B negative.

TABLE 4 *C. difficile* stool culture results for group 4, cohort 1

Group and subject no.	Stool culture result[a]														
	Predose[b] (day −6)	Study drug administration days												Follow-up days	
		2	3	4	5	6	7	8	9	10	11	12	13	14	21	28
Placebo																
14103	−	−	−	−	−	−	−	−	−	−	+ (p)	−	+ (p)	+ (p)	+ (p)	−
14107	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14111	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
VP20621 (10^4 spores)																
14101	−	−	−	−	+ (n)	+	+	+	+	+	−	+	+	+ (n)	−	−
14102	−	+ (n)	+	+	+	+	+	−	+	+	+	+	+	+ (n)	+ (n)	+ (n)
14104	−	−	−	−	+ (n)	+	+	−	+	+	−	+	+	+ (n)	−	−
14105	−	−	+ (n)	+	+	+	+	−	+	+	+	+	+	+ (n)	+ (n)	+ (n)
14106	−	−	+ (n)	−	+	+	−	+	+	−	−	+	+	+ (n)	−	−
14108	−	−	−	+ (n)	+	+	−	+	+ (n)	−	−	−	−	−	+ (n)	+ (n)
14109	−	−	−	+ (n)	+	+	+ (n)	−	+	+	+ (n)	+	+ (n)	+ (n)	+ (n)	−
14110	−	−	−	+ (n)	+	+	−	+	+	+	−	+	+	+ (n)	−	−
14112	−	−	+ (n)	−	−	+ (n)	+	+	+	+	−	+	+	+ (n)	−	−

[a] Symbols and abbreviations: −, *C. difficile* culture negative; +, *C. difficile* culture positive; (n), toxin A/B negative; (p), toxin A/B positive; shaded cells, the isolate was confirmed to be consistent with VP20621.
[b] Predose samples were collected prior to start of pretreatment with oral vancomycin.

flatulence, mild gingival bleeding, mild oral paresthesia, mild toothache, and moderate vomiting. After randomization and treatment with study drug, all AEs in group 4 were of mild intensity, and there was no evidence that the type or severity of events was dose dependent. The only non-GI event reported by more than one VP20621-treated subject in group 4 was rash (2 subjects at the 10^6 dose). The events were reported as "slight erythema with pruritus, both forearms" and "erythematous rash, both legs and arms"; both events were mild, and neither required treatment. A summary of all GI events in group 4 is presented in Table 7. Diarrhea or loose/watery stools were reported in 3/27 (11%) VP20621-treated subjects. All 3 episodes were mild and transient (one episode of watery stools on day 6, one episode of loose stools on day 8, and intermittent [two episodes] watery stools on day 8); all resolved, despite continued dosing to day 14, and none required treatment.

Notably, the safety profile of the five subjects in group 4 who received placebo and had positive *C. difficile* stool culture results was unremarkable. There were no reports of diarrhea or loose/watery stools in any of these subjects (including the two subjects with isolates that were toxin A/B positive). The only GI symptom was abdominal distension in one subject (10^8 dose), but this event

TABLE 5 *C. difficile* stool culture results for group 4, cohort 2

Group and subject no.	Stool culture result[a]														
	Predose[b] (day −6)	Study drug administration days												Follow-up days	
		2	3	4	5	6	7	8	9	10	11	12	13	14	21	28
Placebo																
14204	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14207	−	−	−	−	−	+ (p)	+	+	+	+	+	−	−	+ (p)	−	−
14210	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
VP20621 (10^6 spores)																
14201	−	+ (n)	−	−	−	+	+	+	+	+	+	+	+	+ (n)	−	−
14202	−	−	−-	−	+ (n)	+	+	+	+	+	+	−	+ (n)	−	+ (n)	−
14203	−	+ (n)	−	−	+	+	−-	+	+	+	+	+	+	+ (n)	−	−
14205	−	+ (n)	−	+	+	+	+	+	+	+	+	+	+	+ (n)	NA	+(n)
14206	−	−	−	−	−	+ (n)	+	+	+	+	+	+	+	+ (n)	−	−
14208	−	−	−	−	+ (n)	+	+	+	+	+	+	+	+ (n)	−	−	−
14209	−	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+ (n)	+ (n)	−
14211	−	−	−	−	+ (n)	+	+	+	+	−	+	+	+	+ (n)	−	−
14212	−	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+ (n)	+ (n)	−

[a] Symbols and abbreviations: −, *C. difficile* culture negative; +, *C. difficile* culture positive; (n), toxin A/B negative; (p), toxin A/B positive; NA, stool sample not available; shaded cells, the isolate was confirmed to be consistent with VP20621.
[b] Predose samples were collected prior to start of pretreatment with oral vancomycin.

TABLE 6 *C. difficile* stool culture results for group 4, cohort 3

Group and subject no.	Stool culture result[a]														
	Predose[b]	Study drug administration days												Follow-up days	
	(−6)	2	3	4	5	6	7	8	9	10	11	12	13	14	21	28
Placebo																
14303	−	−	−	−	+ (n)	+	+	+	+	+ (n)	−	−	−	−	−	−
14307	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+	+ (n)	−	−
14310	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+	+ (n)	+ (n)	+ (n)
VP 20621 (10^8 spores)																
14301	−	+ (n)	−	+	+	+	+	+	−	+	+	+	+	+ (n)	−	−
14302	−	−	+ (n)	−	+	+	+	+	+	+	+	+	+	+ (n)	−	−
14304	−	+ (n)	+	+	+	+	+	+	+	+	+	+	+	+ (n)	−	−
14305	−	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+ (n)	−	+ (n)
14306	−	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+ (n)	−	−
14308	−	−	+ (n)	+	+	+	+	+	+	+	+	+	+	+ (n)	+ (n)	−
14309	−	−	−	+ (n)	+	+	−	+	+	+	+ (n)	−	−	−	+ (n)	−
14311	−	−	−	+ (n)	+	+	+	+	+	+	+	+	+	+ (n)	−	−
14312	−	−	−	+ (n)	+	+	+	+	+	+	+	+	−	+ (n)	+ (n)	−

[a] Symbols and abbreviations: −, *C. difficile* culture negative; +, *C. difficile* culture positive; (n), toxin A/B negative; (p), toxin A/B positive; shaded cells, the isolate was confirmed to be consistent with VP20621.
[b] Predose samples were collected prior to start of pretreatment with oral vancomycin.

started during pretreatment with vancomycin. All AEs in these subjects were mild and resolved during study drug dosing to day 14.

DISCUSSION

VP20621 was well tolerated at all doses administered. There were no serious or severe AEs, and no subjects were discontinued from study drug due to an AE. In group 4, during pretreatment with oral vancomycin, diarrhea or loose/watery stools were reported by 7% of subjects. During dosing with study drug, all subjects who received VP20621 became stool culture positive, and 11% had loose/watery stools; all three cases were mild and transient (occurring on day 6 or day 8) and resolved spontaneously without treatment. Given the similar rates of diarrhea or loose/watery stools during vancomycin before treatment and after treatment with study drug, these events may be associated with the underlying disruption of the intestinal microbiota after antibiotic treatment. Appropriate vigilance for monitoring of diarrhea events remains necessary for future VP20621 clinical trials, but the infrequent GI events in this study and the absence of a relationship to VP20621 dose are reassuring.

TABLE 7 Gastrointestinal AEs in group 4

Group and AE	No. (%) of subjects				
		VP20621			
	Placebo ($n = 9$)	10^4 spores ($n = 9$)	10^6 spores ($n = 9$)	10^8 spores ($n = 9$)	All doses ($n = 27$)
Subjects with ≥1 AE	5 (56)	5 (56)	6 (67)	1 (11)	12 (44)
Subjects with ≥1 GI AE	3 (33)	4 (44)	2 (22)	0	6 (22)
Diarrhea	0	2 (22)	1 (11)	0	3 (11)
Dyspepsia	1 (11)	1 (11)	1 (11)	0	2 (7)
Abdominal discomfort	0	0	1 (11)	0	1 (4)
Abdominal pain upper	0	0	1 (11)	0	1 (4)
Constipation	0	1 (11)	0	0	1 (4)
Flatulence	2 (22)	0	0	0	0
Gingival pain	1 (11)	0	0	0	0

In addition to the safety data obtained in this phase 1 trial of VP20621, an additional noteworthy observation was the detection of *C. difficile* in the stool of subjects who had not received prior antimicrobial treatment. This occurred in group 3 (10^8 spores twice daily for 5 days) and did not persist beyond day 7, suggesting that this was transient pass-through of ingested *C. difficile* rather than colonization. This is the first human observation that *C. difficile* detection in stool following ingestion of spores may not necessarily reflect gastrointestinal colonization. This possibility has been speculated previously, without conclusive evidence.

A second observation of interest was the duration of colonization by subjects pretreated with vancomycin and given VP20621 spores. In each dosage cohort of group 4, 4 of 9 subjects had persistent detection of VP20621 at day 21 or 28, suggesting that longer-term colonization had occurred and was independent of the dose administered. Variability in the time to first detection of *C. difficile* in stool was also observed, ranging from 2 to 6 days. It is presumed that the primary determinant of the time required for stool detection is the amount of residual vancomycin remaining in the stool and that spores that vegetate will not survive until the stool level of drug is below the MIC of *C. difficile* (1).

An unexpected observation was the detection of toxin-positive *C. difficile* in the stool of group 4 placebo subjects pretreated with vancomycin. This occurred persistently in 2 of 6 placebo patients in the two lower-dose cohorts. Neither subject developed symptoms, but the risks of ingestion of and colonization by wild-type *C. difficile* following any antimicrobial exposure were convincingly demonstrated. These strains were most likely acquired from the environment and were able to colonize following disruption of the intestinal microbiota by vancomycin. Similar observations of detection of *C. difficile* or its toxins in stool have been made in phase 1 testing of cephalosporin antibiotics (4, 5, 9). In the highest-dose cohort in group 4 (10^8 spores), the three placebo subjects became

colonized with VP20621 as rapidly as some subjects who were given spores. This is likely due to exposure to VP20621 spores within the study site through contact with the other study subjects or through contact with items within the shared living facilities. Since the frequency and amount of exposure of these patients to spores on a daily basis are unknown, it is of interest that each had persistent *C. difficile* detected in their stools for 6, 11, and 25 days, respectively, indicating that at least one of these patients remained colonized well beyond the time of residence and exposure in the facility.

The unintended acquisition of VP20621 spores by subjects who were given placebo in the phase 1 facility raises the possibility of the similar dissemination of spores in a health care environment if large numbers of patients are being treated with NTCD spores to prevent primary and recurrent CDI. It is clear that transmission of NTCD strains is already occurring naturally in health care facilities (12), but deliberate administration of these strains could tip the transmission balance in favor of NTCD over toxigenic strains. In effect, a critical level of use of NTCD could result in establishment of herd protection from the combination of deliberate administration and inadvertent transmission of NTCD. Whether such an event is likely to occur will await results of clinical trials in health care facilities.

Limitations of the study include the fact that all subjects were Caucasian, that the small cohorts of younger subjects received single doses, and only older volunteers participated in groups 3 and 4. A possible limitation is the use of a mildly sporicidal disinfectant (Perform classic concentrate OXY; Schülke & Mayr GmbH, Norderstedt, Germany) for daily cleaning of rooms and surfaces, which could have allowed *C. difficile* spores to survive. However, the subjects in each study group spent considerable time together in common areas, providing ample opportunity for direct spore transmission.

This phase 1 study revealed that viable NTCD was detected in stool cultures at one or more time points in all subjects who received multiple doses of VP20621, with few GI AEs. These encouraging data suggest that the VP20621 strain of *C. difficile* may be able to colonize the GI tract of patients with disrupted GI microbiota who are at risk for acquiring toxigenic *C. difficile* and potentially prevent both primary and recurrent CDI. A phase 2 dose-ranging and safety trial of VP20621 strain M3 for prevention of recurrent CDI in patients with CDI is being conducted.

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REFERENCES

1. **Cadnum J, et al.** 2011. Defining the vulnerable period for re-establishment of *Clostridium difficile* colonization after treatment with oral vancomycin, abstr. 409. Abstr. Soc. Healthcare Epidemiol. Am. Annu. Scientific Meeting, Dallas, TX.
2. **Clabots CR, et al.** 1993. Development of a rapid and efficient restriction endonuclease analysis typing system for *Clostridium difficile* and correlation with other typing systems. J. Clin. Microbiol. **31**:1870–1875.
3. **Borriello SP, Barclay FE.** 1985. Protection of hamsters against *Clostridium difficile* ileocaecitis by prior colonisation with non-pathogenic strains. J. Med. Microbiol. **19**:339–350.
4. **Finegold SM, et al.** 1987. Bowel flora changes in humans receiving cefixime (CL 284,635) or cefaclor. Antimicrob. Agents Chemother. **31**:443–446.
5. **Iwata S, et al.** 1991. The influence of cefpirome on intestinal bacterial flora. Jpn. J. Antibiot. **44**:62–83.
6. **Kyne L, Warny M, Qamar A, Kelly CP.** 2001. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. Lancet **357**:189–193.
7. **Kyne L, Warny M, Qamar A, Kelly CP.** 2000. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N. Engl. J. Med. **342**:390–397.
8. **Merrigan MM, Sambol SP, Johnson S, Gerding DN.** 2003. Prevention of fatal *Clostridium-difficile*-associated disease during continuous administration of clindamycin in hamsters. J. Infect. Dis. **188**:1922–1927.
9. **Motohiro T, et al.** 1986. Effect of cefminox on bacterial flora in human adult feces. Jpn. J. Antibiot. **39**:909–957.
10. **Sambol SP, Merrigan MM, Tang JK, Johnson S, Gerding DN.** 2002. Colonization for the prevention of *Clostridium difficile* disease in hamsters. J. Infect. Dis. **186**:1781–1789.
11. **Seal D, et al.** 1987. Treatment of relapsing *Clostridium difficile* diarrhoea by administration of a non-toxigenic strain. Eur. J. Clin. Microbiol. **6**:51–53.
12. **Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN.** 1998. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. Lancet **351**:633–636.
13. **Tatarowicz W, et al.** Safety and tolerability of an oral suspension of VP20621, spores of a non-toxigenic *C. difficile* strain; first in human administration to healthy adult subjects, abstr. PIII-17. Abstr. 10th Biennial Congr. Anaerobe Soc. Am.
14. **Villano SA, et al.** 2010. Phase I evaluation of an oral suspension of VP20621, spores of a non-toxigenic *C. difficile* strain (NTCD), in healthy older subjects pretreated with vancomycin, abstr. F1-2127b. Abstr. 50th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
15. **Wilson KH, Sheagren JN.** 1983. Antagonism of toxigenic *Clostridium difficile* by nontoxigenic *C. difficile*. J. Infect. Dis. **147**:733–736.