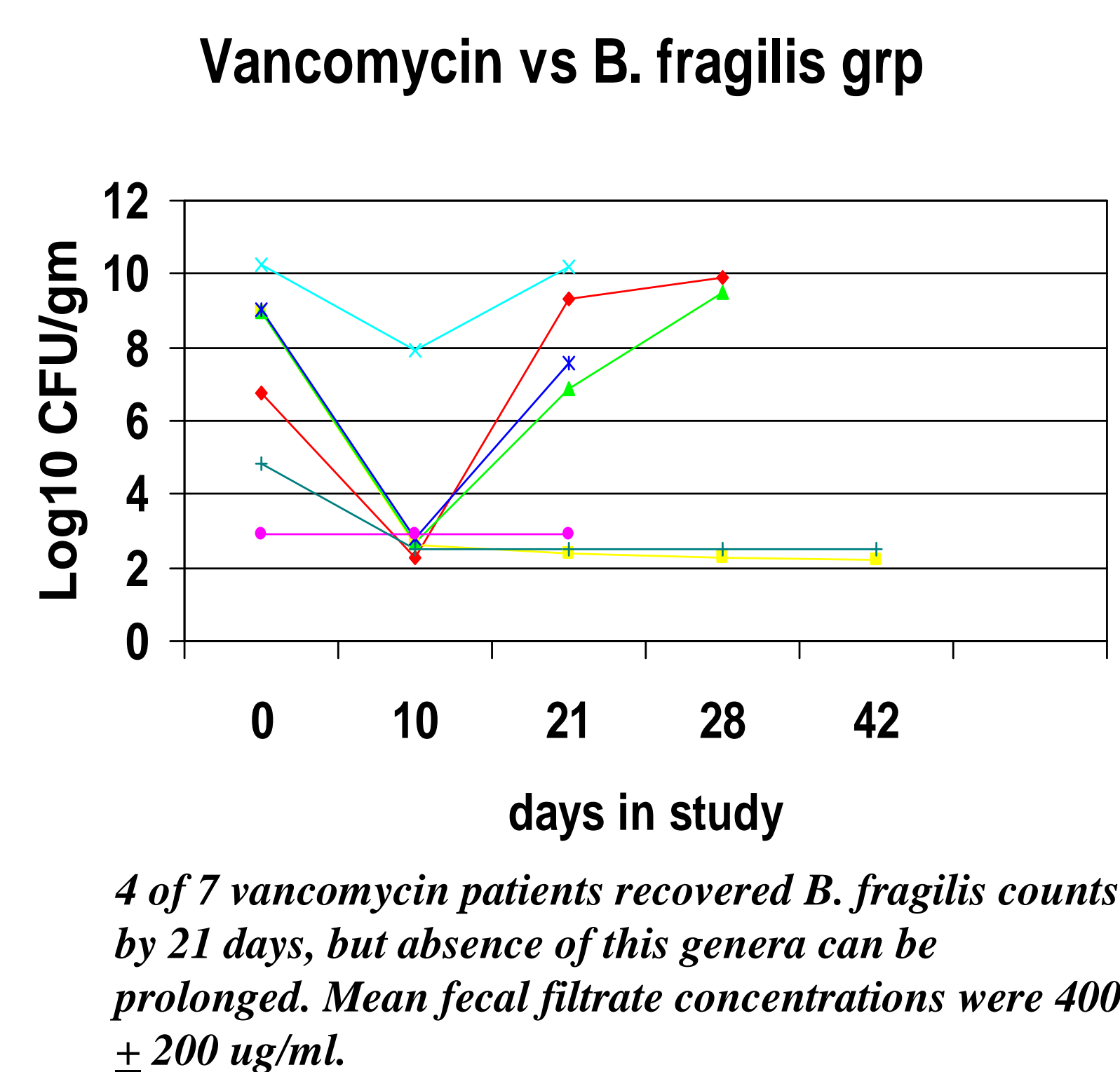
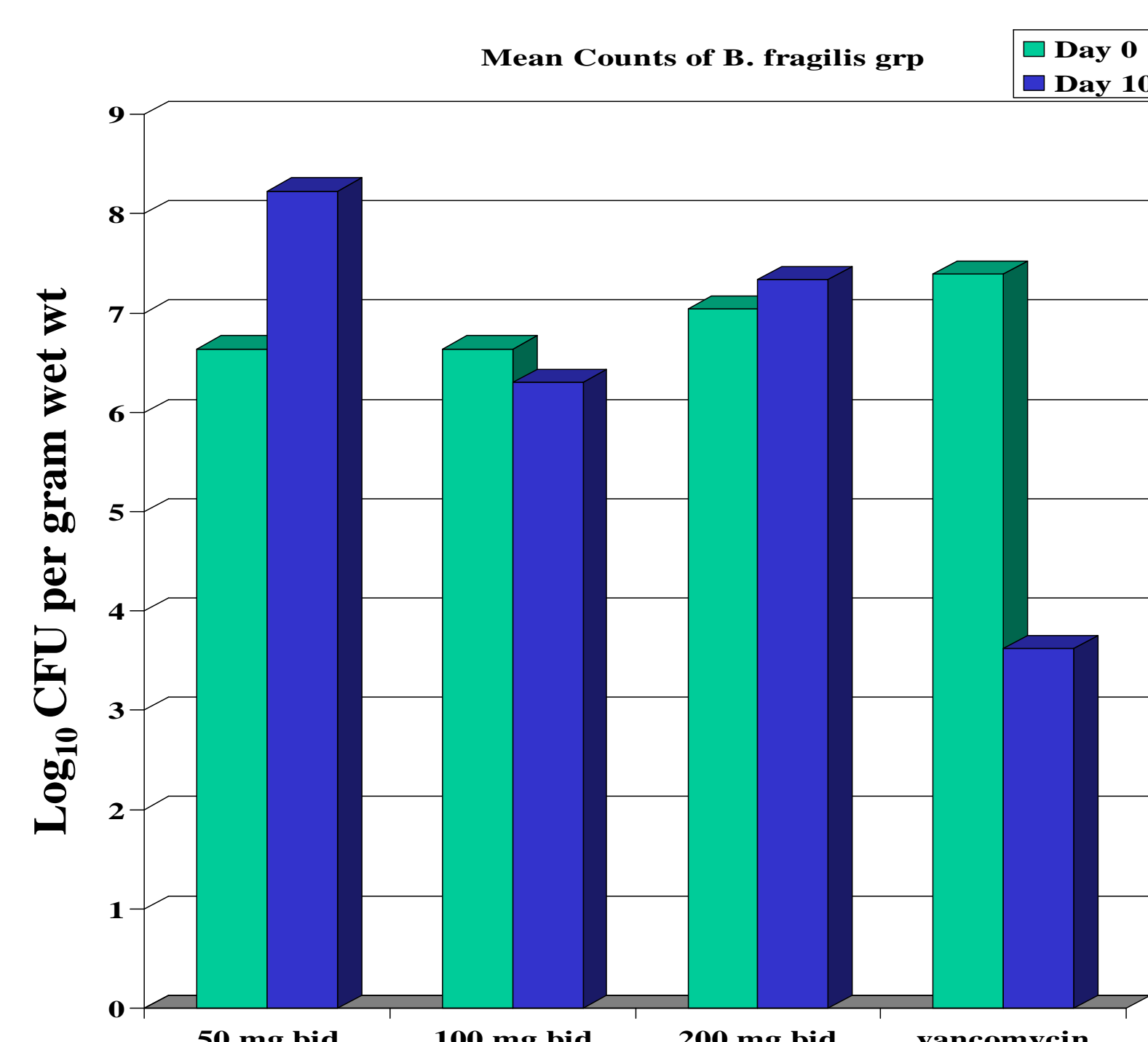


PAR-101 is selectively effective against *Clostridium difficile* in-vivo and has minimal effect on the anaerobic fecal flora. T.J. Louie, J. Emery, W. Krulicki, B. Byrne, M. Mah. University of Calgary, Alberta, Canada

ABSTRACT

Background. Optimal antibacterial therapy of *C. difficile* diarrhea (CDD) should require the reduction of pathogen counts, yet spare remaining anaerobic flora. To determine whether PAR-101 is selective, serial stool samples were collected at study entry & at day 10 during the conduct of a phase 2a dose ranging study of CDD treatment. **Methods:** Patients (n=32) were randomized to receive 50, 100 or 200 mg twice daily of PAR-101 for 10 days. No prior therapy was given to 24 patients; 8 receive 1 or 2 doses of standard therapy. As ecologic controls, 7 additional patients were treated with vancomycin 125 mg qid for 10 days. Fresh stool samples were cultured $10^{-2.4,6,8}$ for *C. difficile* on CCFA media. At study entry and day 10, aerobic and anaerobic fecal flora cultures, diluted $10^{3.5,7,9}$, were examined for major flora shifts. Since *Bacteroides* group organisms are ubiquitous and cultivable, this genera was selected as an indicator of the integrity of the microbial flora. **Results:** At study entry, mean \log_{10} CFU \pm SD / g of *C. difficile* (all PAR-101 patients) were 6.8 ± 3.6 , range 2-10.9. At day 10, with the exception of one patient receiving 50 mg, all other patients had *C. difficile* counts < 2 . Vancomycin was similarly effective. *Bacteroides* group counts at study entry were $< 3, 3.8, & 8.5$ \log_{10} CFU/g in 1/3 each of patients. Mean \pm SD of \log_{10} CFU/g of *Bacteroides* group counts, Days 0/10 were: PAR-101 50 mg (n=10) $6.6 \pm 2.8 / 8.2 \pm 2.6$ (p=0.11, wilcoxon matched pairs signed-ranks test, 2 tailed); PAR-101 100 mg (n=8) $6.6 \pm 2.8 / 6.3 \pm 2.5$ (p=0.44); PAR-101 200 mg (n=11) $7.0 \pm 2.8 / 7.3 \pm 3.1$ (p=0.56); and vancomycin (n=7) $7.4 \pm 2.7 / 3.6 \pm 1.9$ (values $< 3 \log_{10} = 2.9$) (p=0.03). **Conclusion:** Patients with CDD have variably impaired normal flora, about 1/3 of which is severe. PAR-101 effectively eradicates *C. difficile*. A dose-dependent reduction in *Bacteroides* counts was not observed. Vancomycin significantly reduces *Bacteroides* counts during CDD treatment. PAR-101 appears to meet criteria as a treatment for *C. difficile* diarrhea.



Mean \pm SD of \log_{10} CFU of *Bacteroides* grp counts** / g feces

	PAR 101 50 mg BID (n=10)	PAR 101 100 mg BID (n=8)	PAR 101 200 mg BID (n=11)	VANCOMYCIN 125 mg QID (n=7)
DAY 0 (study entry)	6.6 \pm 2.8	6.6 \pm 2.8	7.0 \pm 2.9	7.4 \pm 2.7
DAY 10	8.2 \pm 2.6	6.3 \pm 2.5	7.3 \pm 3.1	3.6 \pm 1.9**
p value Wilcoxon matched pairs signed rank test, 2 tailed.	0.11	0.44	0.56	0.03

** counts $< 3 \log_{10} = 2.9$

INTRODUCTION

While metronidazole or vancomycin treatment of *Clostridium difficile* associated diarrhea (CDAD) is effective in ~85-90% of cases, a relapse rate of 16-21% is a serious clinical deficiency. In addition, recent clinical experience with metronidazole as initial therapy of CDAD has raised concerns regarding sub-optimal clinical responses and higher than anticipated relapse rates. High vancomycin concentrations in the fecal biomass are thought to contribute to suppression of the normal flora, accounting for relapsing disease. PAR-101, (formerly OPT-80) was originally shown in 1991 to durable cure lethal *Clostridium difficile* disease in Syrian Hamsters. Recent development of this macrocyclic antibiotic has shown that PAR-101 is

- Well tolerated in human volunteers.
- ~10 fold more active than vancomycin against *C. difficile* in-vitro (MIC90 0.12-0.25 ug/ml for OPT-80 versus 2.0 for vancomycin).
- Associated with a long PAE (Post-Antibiotic Effect).
- Poorly active against gram-negative members of the anaerobic fecal flora in-vitro (*Bacteroides fragilis* group MIC90 > 1024 ug/ml for OPT-80 versus 128 ug/ml for Vancomycin).

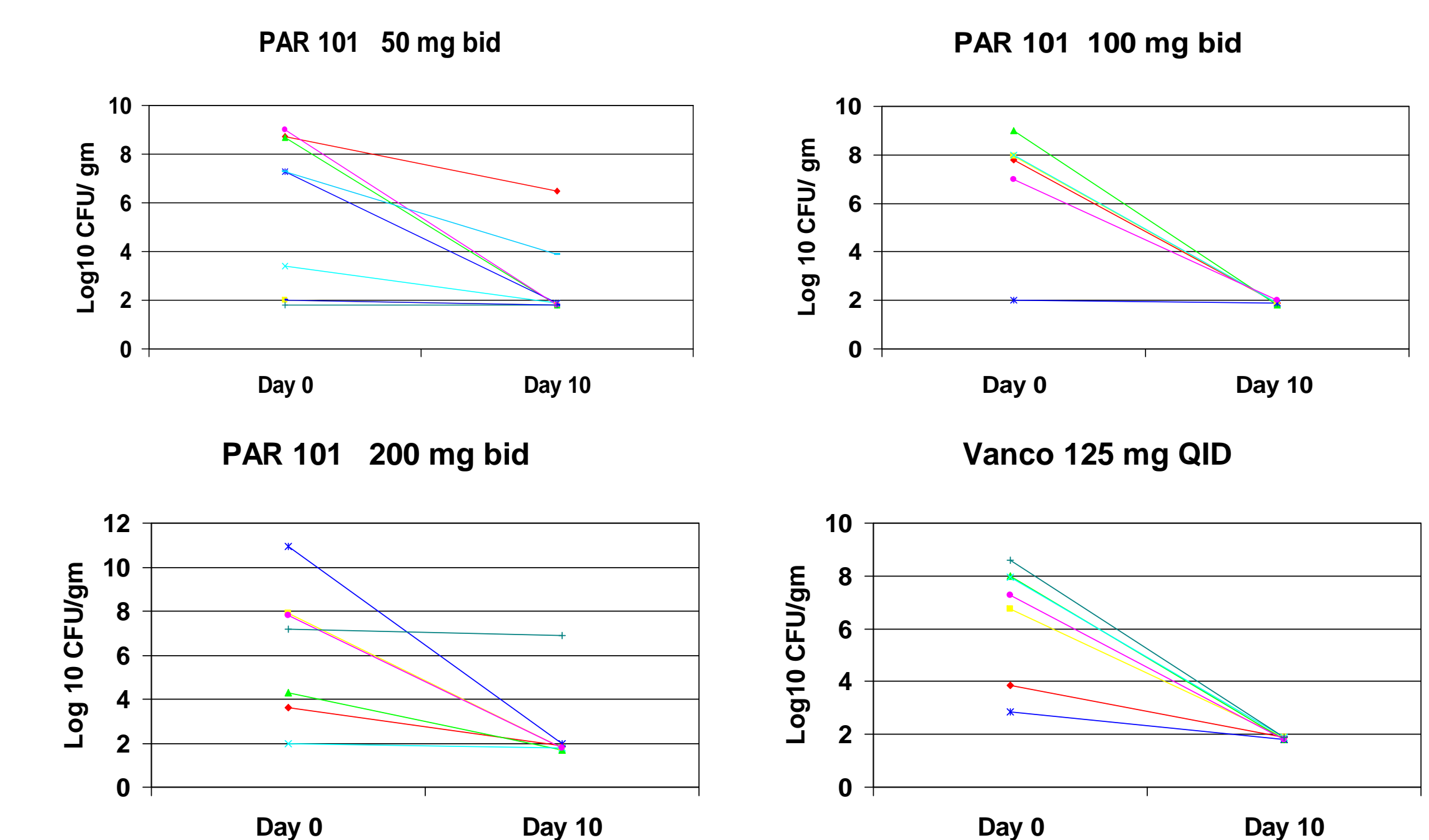
OBJECTIVE

To test the hypothesis that PAR-101 is selectively active in-vivo against *C. difficile* and could be relatively sparing of the normal anaerobic fecal flora, quantitative stool cultures were performed on serial stool samples obtained from patients entered into a Phase 2a dose ranging clinical trial of OPT-80 (now designated PAR-101). Optimal antibiologic therapy of *C. difficile* diarrhea should eradicate the vegetative forms of the pathogen, yet spare major components of the normal flora presumed to be responsible for colonization resistance.

METHODS

- Single center study in Calgary Health Region catchment area, population ~1 million
- Randomized open label, dose ranging Phase 2a study comparing 50 mg, 100 mg or 200 mg Q 12 hourly of PAR-101 for 10 days p. o. as therapy of CDAD.
- Following completion of the trial recruitment, a separate ecologic control group of patients who otherwise would be eligible for the trial were treated with Vancomycin 125 mg QID for 10 days as a treatment / ecologic control.
- Mild to moderate CDAD: >3 but < 12 diarrheal samples / 24 hours at study entry, positive *C. difficile* toxin assay, fever < 39 degrees C, WBC $< 30,000$ /mm³, no vomiting, no severe abdominal discomfort
- Primary CDAD or first relapse episode only.
- Treatment naïve if possible. The protocol allowed up to 3 prior doses of standard therapy, but for this evaluation, a maximum of 2 doses of standard therapy was allowed. In this study population, 24 patients were treatment naïve
- No concomitant parenteral antibiotic therapy for any condition.
- Serial stool samples: in addition to the original diagnostic sample, a repeat collection of stool > 5 grams (10-30 grams usually) was obtained at study entry, at day 4, 7, 10, 14, 21, 28 and 42 days after study entry
- For this report, results of day 0 and day 10 stools are compared for changes in *C. difficile* counts and in counts of major genera of the normal colonic flora.
- C. difficile* quantitative counts and fecal filtrate concentrations of *C. difficile* cytotoxin B by HeLa cell assay were determined with freshly passed samples as refrigeration is deleterious to determination of quantitative counts of *C. difficile*.
- Since *Bacteroides* group organisms are considered to be uniformly present in subjects and in high counts, and is likely one of the major components of the normal flora conferring 'colonization resistance', this group was used as an index of suppression of the anaerobic fecal flora. For patients who failed to show return of the *Bacteroides* group species at 10 days, subsequent samples were processed to document time of return of this group. If samples were not immediately processed, aliquots were frozen at -80 degrees C with 15% glycerol / Brain Heart Infusion Broth for subsequent processing.
- Media and methods for anaerobic flora cultures is based on the Wadsworth-KTL Anaerobic Manual, 6th ed, 2002. *C. difficile* counts were determined by dilution of the sample $10^{-2,4,6,8}$ / gram stool wet weight on CCFA agar. Spore counts were determined by treating an aliquot of stool with an equal volume of 100% ethyl alcohol x 1 hour, centrifuged, washed twice and resuspended for quantitative counts.
- Normal flora cultures were quantified by dilution $10^{-3,5,7,9}$ using MacConkey, BAP, m-Enterococcus agar, Lab M anaerobic blood agar, BAP, BBE, KVLB, PEA agars incubated for 48 hours before initial inspection, and further incubated for up to 7 days.
- For vancomycin ecologic controls, vancomycin fecal filtrate concentrations were determined in triplicate by bioassay using a *C. perfringens* as the indicator organism.
- Differences in microbial counts were determined after \log_{10} transformation using wilcoxon matched pairs signed-ranks test, 2 tailed. For counts $< 3 \log_{10}$, a value of 2.9 was used.

Quantitative reduction of *C. difficile* vegetative counts



Conclusions

- Based on quantitative *Bacteroides* group counts, patients with *C. difficile* diarrhea have variably impaired normal flora at study entry, with approximately 1/3 in the $3 \log_{10}$ CFU/gm range, 1/3 in counts of 4-7 \log_{10} CFU, and the remainder with higher counts (none in the normal range of 11-12 \log_{10} CFU).
- All dosages of PAR-101 appeared to reduce counts of *C. difficile*, as did vancomycin.
- A dose dependent reduction in *Bacteroides* counts with increasing dosages of PAR-101 was not observed.
- Vancomycin severely impairs *Bacteroides* counts during therapy and although most patients recover their counts, a minority have prolonged absence.
- Based on these data and clinical outcomes showing a high response rate accompanied by a low relapse rate, it would appear that the 200 mg dose of PAR-101 would be an appropriate dosage to undergo further clinical investigation.

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