Efficacy of Germinants and Omadacycline for Preventing *Clostridioides difficile* Relapse in a Murine Model

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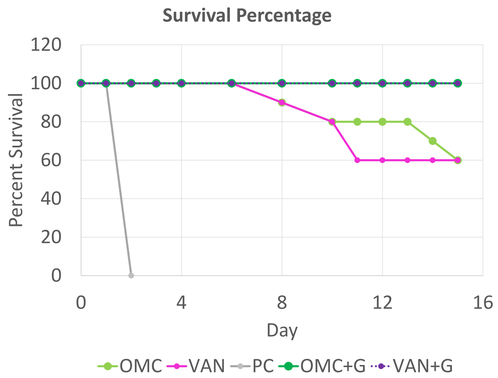
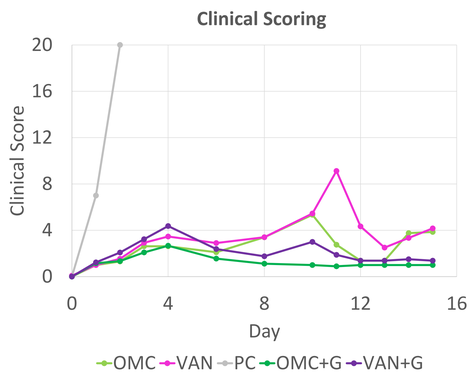
Introduction

- *Clostridioides difficile* (Cd) is labeled one of five urgent level pathogens by the CDC. The urgency is related to the high burden of disease, limited effective antimicrobials, and recurrent *C. difficile* infections (rCDI) from residual spores.
- Impervious to antibiotics, *C. difficile* spores can be transformed into vegetative cells by germinants for antibiotic targeting *in vivo*.
- Taurocholate, an endogenous bile acid, is the main germination signal and co-germinants, divalent cations and amino acids, increase germination rates. Previously *in vitro* we found that docusate i) enhances germination, likely through removal of the spore's exosporium, and ii) helps solubilize spores, which may aid in removal from gastrointestinal epithelial tissue in vivo. [1]
- This study aims to evaluate spore reservoir eradication through applying germinants with antibiotics in mice.

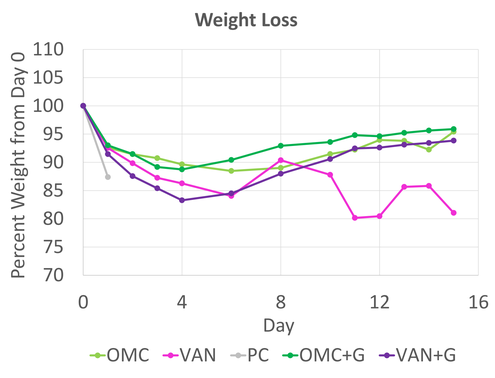
Results



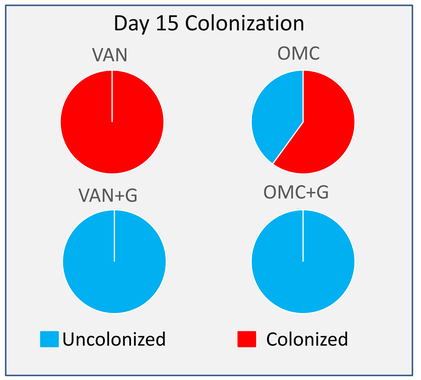
❖ Germinant administration improved day 15 survival.



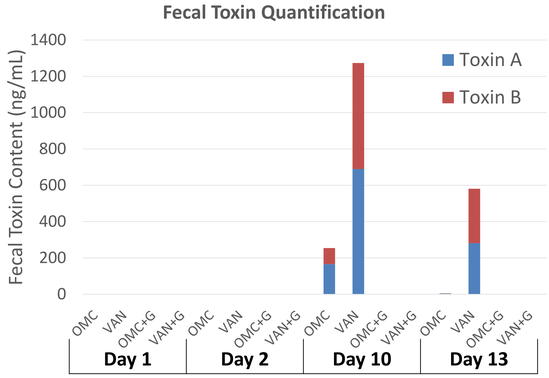
❖ Germinants did not result in increased clinical scoring during administration and reduced scores by day 15.



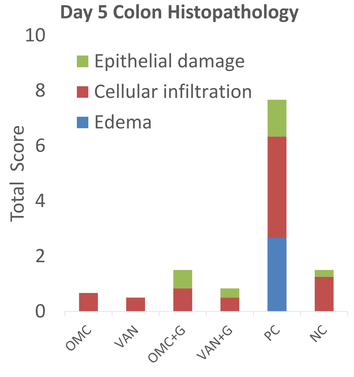
❖ Germinants did not result in increased weight loss during administration and prevented loss on day 15.



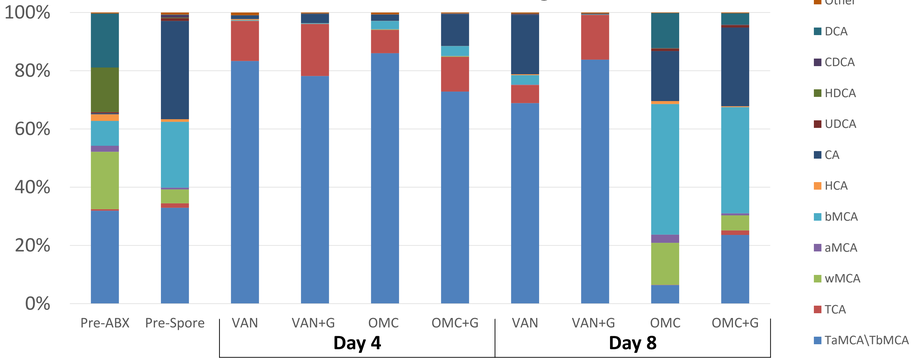
❖ Germinants had no detectable Cd colonization at day 15, OMC had 60%, and VAN had 100%.



❖ Germinants did not cause significant early toxin production and prevented production during clindamycin administration.

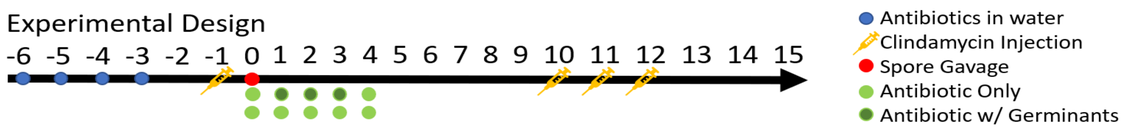


❖ Colon histopathology was similar between infected and non-infected groups at day 5.



❖ Antibiotic water diminished bile acid diversity. More diversity returned to OMC and OMC+G by day 8 than VAN and VAN+G. Germinant administration did not appear to affect the bile acid composition the following day.

Methods

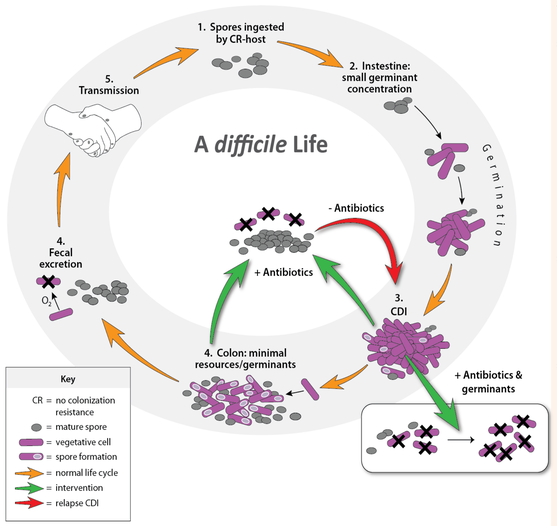


A published murine model of rCDI using C57BL/6 mice and 1 x 10^5 *C. difficile* spores (VPI 10463) with modification was used as shown above.[2]

Six hours after inoculation, mice received 1.5 mg vancomycin (VAN, n=10) or 0.25 mg omadacycline (OMC, n=10) daily by oral gavage until day 4 or either with germinant (G) solution (8 mg of sodium taurocholate, 10 mg of taurine, 0.2 mg of sodium docusate, and 1.72 mg of calcium gluconate) given concomitantly on days 1 to 3 (OMC+G, n=9 and VAN+G, n=8). As a positive control (PC), five mice did not receive antibiotics after spores. As a negative control (NC), five mice were given initial antibiotics but no spores. To induce rCDI, clindamycin was given on days 10 to 12. Complete cage changes occurred on days 0 and 3 for each group.

Survival was recorded daily. **Clinical scoring** included six categories with higher scores representing severe disease: percent weight loss from day 0, posture, activity, fur appearance, eye appearance, and stool consistency. Scoring was assessed by veterinary staff except for weight loss and stool consistency. Mice with scores ≥14 were euthanized. **Weight loss** from day 0 is also shown as a stand-alone figure. Fecal samples from mice with median amounts of weight loss were taken directly from each mouse to measure **toxin production** (n=5/group) by sandwich ELISA, **bile acid** content (n=2/group) through the University of Michigan Biomedical Research Core Facilities, and day 15 colonization by culturing methods. Toxin production for PC mice is omitted for clarity. Mice that died prior to day 15, were too sick to provide samples, or had positive stool culture were considered **colonized on day 15**. Three additional mice in OMC, VAN, OMC+G, and VAN+G followed the same experimental design but were euthanized on day 5 along with 2/5 NC mice for **colon histopathology**. In addition, 3 mice from the PC group were evaluated. Colons were excised and stored in formalin cassettes for evaluation by a veterinary pathologist. The pathologist was blinded to all slides except for the NC and PC mice.

Conclusions



General concepts of the impact of this study (**bottom right**) in the CDI cycle are shown in the figure, "A *difficile* Life."

- Germinant and antibiotic combinations improved survival in an rCDI mouse model compared to antibiotics alone. Germinants did not induce toxin production, cause clinical deterioration, or increase weight loss. Day 15 colonization was not detected in any germinant treated mice, suggesting spore reservoir elimination.
- OMC and OMC+G groups had more bile acid diversity on day 8, signaling less microbiome dysbiosis compared to vancomycin.
- While this data is promising, further study in murine models with more contemporary *C. difficile* strains is needed. This provides basis for further study of germinants combined with antibiotics to reduce rCDI.

[1]Budi N, Godfrey JJ, Safdar N, Shukla SK, Rose WE. Omadacycline compared to vancomycin when combined with germinants to disrupt the life cycle of *Clostridioides difficile* [published online ahead of print, 2021 Mar 1]. *Antimicrob Agents Chemother*. 2021;65(5):e01431-20. doi:10.1128/AAC.01431-20
[2]Chen X, Katchar K, Goldsmith JD, et al. A mouse model of Clostridium difficile-associated disease. *Gastroenterology*. 2008;135(6):1984-1992. doi:10.1053/j.gastro.2008.09.002
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