An osmotic laxative renders mice susceptible to prolonged *Clostridioides difficile* colonization and hinders clearance

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Abstract

(Modify depending on target journal) Antibiotics are a major risk factor for *Clostridioides difficile* infections (CDIs) because of their impact on the intestinal microbiome. However, non-antibiotic medications such as the ubiquitous osmotic laxative polyethylene glycol (PEG) 3350, also alter the microbiota, but whether PEG impacts CDI susceptibility and clearance is unclear. To examine how PEG impacts susceptibility, we treated C57Bl/6 mice with 5-day and 1-day doses of 15% PEG in the drinking water and then challenged the mice with *C. difficile* 630 spores. We used clindamycin-treated mice as a control because they consistently clear *C. difficile* within 10 days post-infection (dpi). To examine how PEG treatment impacts clearance, we administered PEG for 1 day to clindamycin-treated, *C. difficile*-challenged mice either immediately following challenge or 3 dpi. We collected longitudinal stool samples to examine *C. difficile* levels in the stool via anaerobic culture and profiled the microbiota by 16S rRNA sequencing. PEG treatment alone was sufficient to render mice susceptible to CDI and 5-day PEG-treated mice remain colonized for up to 30 dpi. Additionally, 5-day PEG treated mice remained susceptible to CDI 10-days post treatment. In contrast, 1-day PEG treated mice were transiently colonized, clearing *C. difficile* within 7 dpi. Although 5-day PEG-treated mice exhibited prolonged *C. difficile* colonization, we saw no difference in histological inflammation between PEG- and clindamycin-treated mice. Additionally, administering PEG to mice after *C. difficile* challenge prolonged colonization up to 30 dpi in mice that received PEG immediately after challenge and 15 dpi in mice that received PEG 3 dpi. When we examined microbiota composition across our different treatment groups, we found decreased richness in the PEG-treated mice that exhibited prolonged *C. difficile* colonization. Importantly, there were increased Bacteroides and Enterobacteriaceae and decreased Lachnospiraceae and Oscillibacter in most of the PEG-treated mice with prolonged *C. difficile* colonization. Our findings suggest the osmotic laxative PEG 3350 alters the mouse microbiota and disrupts colonization resistance to *C. difficile*, as well as clearance in mice with a CDI. Considering that most hospitals recommend not performing *C. difficile* testing on patients taking laxatives and laxatives are used when administering fecal microbiota transplants via colonoscopy to patients with recurrent CDIs, further studies are needed to evaluate if laxatives impact human microbiota colonization resistance.

Introduction

Results and Discussion

Conclusions

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Materials and Methods

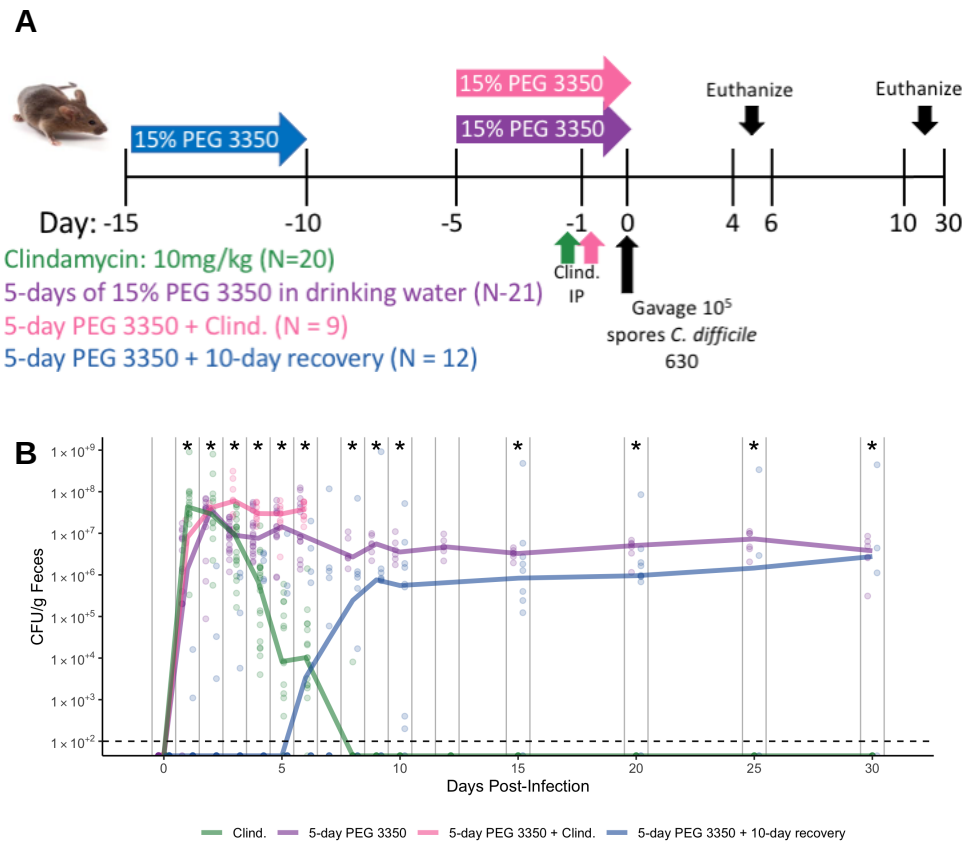

Figure 1. Chronic PEG treatment prolongs susceptibility and mice become persistently colonized with *C. difficile*. A. Setup of the experimental timeline for subset of experiments with 5-day PEG treated mice. B. *C. difficile* CFU/gram stool measured over time (N = 4-`(insert variable name)` mice per timepoint) via serial dilutions. The black line represents the limit of detection for the first serial dilution. CFU quantification data was not available for each mouse due to stool sampling difficulties (particularly the day the mcie came off of the PEG treatment) or early deaths. Lines represent the median for each source and circles represent individual mouse samples.

References