PROBIOTICS TO PREVENT INDUCTION OF CLOSTRIDIUM DIFFICILE INFECTION?

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Abstract

Background and Aims

Clostridium difficile infection (CDI) remains a healthcare burden and recurrent CDI (rCDI) still affects up to 20-30% of patients. Probiotics are live microorganisms that confer a host health benefit, but evidence of their efficacy in CDI prevention/treatment is controversial. Non-toxigenic C. difficile (NTCD) have been used successfully used in animals/humans to reduce CDI incidence. The present study aimed to assess efficacy of two probiotics, Lactobacillus casei Shirota (LcS, Yakult) and a local NTCD, in preventing simulated CDI in an in vitro human gut model.

Methods

CD-negative pooled feces from healthy volunteers (459g) was used to inoculate the gut models. Two probiotics, LcS (6.2 x 10^8 CFU) and NTCD (1 x 10^9 spores), were dosed into separate gut models prior to CD inhibition. NTCD was dosed at 1 x 10^9 spores dosing was for 26d, LcS once-daily and NTCD spores once-weekly. Clindamycin (DA, 3.9 mg/L, QID, 7d) was used to disrupt the gut microflora. Gut model contents were assayed for microbiota composition using viable counting techniques and CD cytotoxin production using a Vero cell cytotoxicity assay. Probiotic dosing ceased 14d before the end of the experiment.

Results

LcS dosing resulted in marked increases in lactobacilli and bifidobacterial viable counts. However, during DA dosing these viable counts declined by 4-log CFU/mL. RT027 spore germination and cell proliferation was observed after DA instillation. Interestingly, another cycle of growth/cytostasis was observed after LcS dosing ceased. NTCD did not colonise the gut model prior to DA instillation; spores were quiescent and washed out. During DA instillation, NTCD spores germinated and vegetative cells multiplied, whereas, RT027 spores did not germinate and no cytostasis was produced. NTCD remained vegetative until the end of the experiment and isolated cells retained their non-toxigenic phenotype.

Conclusions

Instillation of NTCD prevented primary CDI in a human gut model, whereas dosing with LcS did not. LcS, if beneficial in antagonism of CD, is unlikely to be due to nutrient/adhesion competition or production of antimicrobials. NTCD may be beneficial not only in treating CDI but also in the prevention of primary infection. Further work with additional CDI-inviting antimicrobials is needed to better understand the protection that NTCD could confer.

Introduction

Despite improved clinical management strategies for CDI, healthcare costs for treating CDI remain high and have been estimated in the USA at $1.1 billion [1-2]. C. difficile hypervirulence has been attributed to ribotypes 027, 078 and 078A due to increased C. difficile virulence [3-4]. The efficacy of probiotics in preventing CDI are poorly understood due to weakly powered trials, therefore this study aimed to evaluate two probiotics: LcS and NTCD in preventing RT027 CDI using a gut model.

Methods

Human gut model of CDI (Figure 1)

- Viable counting of main cultivable gut microflora groups and C. difficile spores and vegetative cells
- Viable cell cytotoxicity assay for semi-quantification of C. difficile cytotoxin activity
- Pooled feces from healthy elderly volunteers (N=5), >65 years of age, was used as the inoculum

Probiotic strains evaluated

- Lactobacillus casei Shirota (6.2 x 10^9 CFU/day) alone (control) and in combination with RT027 (Figure 4 & 5)
- NTCD (spore 10^9 CFU/day) alone and in combination with RT027 (Figure 2)
- RT027 was evaluated alone as a control for all probiotic experiments (Figure 2B)

Experimental design

- Probiotic strains were introduced 7 days before RT027, and 14 days after antibiotic instillation
- Clindamycin (3.9 mg/L, 7d) was used to facilitate CDI in the gut model

A. Effect of lactobacillus casei Shirota on the gut microflora

B. Induction of CDI in the gut models using clindamycin and RT027

C. Can Lactobacillus casei/ Shirota prevent simulated CDI in the gut model?

D. Can NTCD strain RT027 prevent simulated CDI in the gut model?

Results

LcS control (Figure 3)

- Lactobacilli TVC increased after 3 days of LcS dosing
- Clindamycin dosing deleteriously affected the viability of lactobacilli in line with the clindamycin MIC of the indigenous microflora and LcS

RT027 control (Figure 5)

- C. difficile, spore and vegetative cell production was observed following clindamycin dosing

NTCD control (data not shown)

- NTCD spores germinated and growth was observed after clindamycin dosing (data not shown) commenced
- LcS Shirota prevention of RT027 CDI (Figure 4)

- No antagonism of RT027 growth/toxin production was evident after high daily doses of LcS (6.2 x 10^8 CFU)
- Indeed, onset of cessation of LcS dosing, RT027 underwent another cycle of growth/cytostasis production

NTCD prevention of RT027 CDI (Figures 4 & 5)

- NTCD strain appeared to have a competitive advantage over RT027 following DA dosing; only the NTCD population proliferated and RT027 remained quiescent as spores

Conclusions

- NTCD undergo similar growth cycles to virulent C. difficile in a human gut model
- NTCD appeared to have a competitive advantage over RT027 and prevented RT027 spores from germinating after clindamycin dosing
- More work needed to understand:
  - potential inhibition mechanism (nutrient competition and/or production of inhibitory substances) and importance of antimicrobial susceptibility
  - how the antibiotic used to induce CDI may influence the efficacy of NTCD

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