Effects of omadacycline on gut microbiota populations and *Clostridium difficile* germination, proliferation and toxin production in an *in vitro* model of the human gut

Chilton CH[1], Todhunter SL[1], Ewin D[1], Vernon J[1] and Wilcox MH[1,2]

[1] University of Leeds, Leeds, UK

[2]Microbiology, Leeds Teaching Hospitals Trust, UK,

Leeds General Infirmary and University of Leeds
Old Medical School
Thorseby Place
Leeds, LS1 3EX, UK

c.h.chilton@leeds.ac.uk

Introduction

Omadacycline is a potent aminomethycycline antibiotic with activity against Gram-positive bacteria, including MSSA/MRSA and *S. pneumoniae*, Gram-negative bacteria, and atypical bacteria. It is currently in phase 3 clinical trials for acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia. We have used a well validated, clinically reflective model of the human gut to investigate the effects of omadacycline exposure on the normal gut microbiota, and subsequent potential for induction of simulated *C. difficile* infection (CDI).

Methods

A triple stage chemostat gut model was inoculated with a pooled human faecal slurry (n=5) from healthy volunteers (age ≥60 years) and left for 2 weeks to allow bacterial populations to equilibrate. The model was challenged with 10^7 cfu/mL *C. difficile* spores (ribotype 027) on days 14 and 21. Omadacycline instillation (430 mg/L, once daily, for 7 days) commenced on day 21. The model was observed for a further three weeks post-antimicrobial (days 28-49). Gut microbiota populations and *C. difficile* total viable counts and spore counts were enumerated daily by culture on selective and non-selective agars. Toxin was detected by cell cytotoxicity assay (vero cells), and antimicrobial concentrations were measured by large-plate bioassay using *Kocuria rhizophila* ATCC 9341 as the indicator organism.

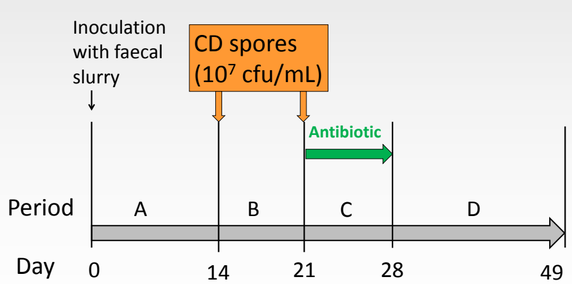

Figure 1 *- Schematic diagram showing the gut model experimental timeline*

Results

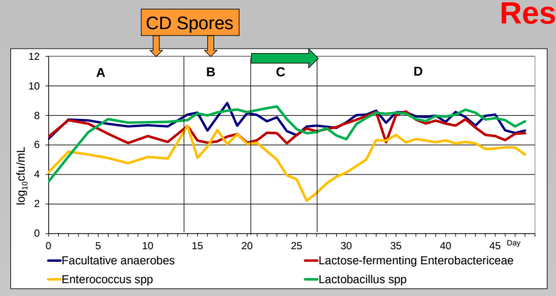

Figure 2A*- Mean facultative anaerobic gut microflora populations (log_{10} cfu/mL) in Vessel 3 of the gut model.*
Periods A-D are defined in Figure 1

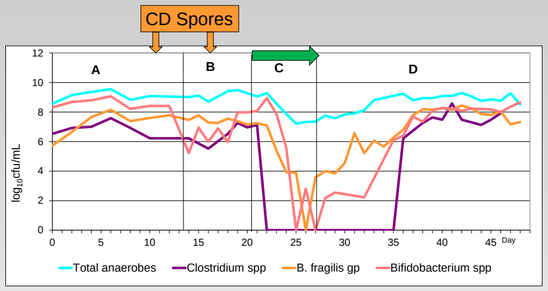

Figure 2B*- Mean obligate anaerobic gut microflora populations (log_{10} cfu/mL) in Vessel 3 of the gut model.*
Periods A-D are defined in Figure 1

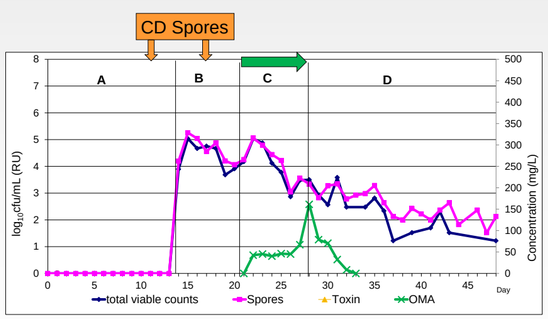

Figure 3*- Mean C. difficile total viable counts and spore counts,(log_{10} cfu/mL), toxin titre and Omadacycline concentration in Vessel 3 of the gut model.*
Periods A-D are defined in Figure 1

Some fluctuation in gut microbiota were observed in the early days of the experiment until a steady state was achieved (Period A, Fig. 2A and 2B. Prior to antimicrobial exposure (Periods A and B), gut microbiota populations were stable (Fig. 2A and 2B). Minor fluctuations in Bifidobacteria populations were observed at the end of period A (Fig. 2B), but these had recovered prior to antibiotic instillation.

Omadacycline instillation caused immediate substantial changes to the microbiota (Fig. 2A and 2B). Declines were observed in populations of;

- Clostridia (~6 log_{10} cfu/mL)
- Bifidobacteria (~6 log_{10} cfu/mL),
- *B. fragilis* grp species (~3 log_{10} cfu/mL),
- *Lactobacillus* spp.(~2 log_{10} cfu/mL)
- *Enterococcus* spp. (~4 log_{10} cfu/mL),

Populations of Enterobacteriaceae remained undisturbed (Fig. 2A).

Omadacycline concentration peaked at ~150mg/L in vessel 2 and vessel 3 (Fig. 2B, V2 data not shown). Higher levels were detected in vessel 1 (~370mg/L, data not shown).

Notably, despite the above disruptions of gut microbiota populations, there was no evidence of simulated *C. difficile* infection following omadacyline exposure. *C. difficile* total viable counts (TVCs) remained roughly equal to spore counts throughout the experiment in all three vessels, indicating that all *C. difficile* remained as spores. There was no vegetative cell proliferation observed. No toxin was detected throughout the experiment in any vessels (Fig. 3).

Discussion

- Despite causing extensive disruption to the gut microbiota, omadacycine exposure did not induce any signs of simulated CDI within the *in vitro* human gut model.
- Simulated CDI in the gut model is characterised by a detectable vegetative cell population (an increase in total viable counts over spore counts), and detectable toxin - typically of 3 or 4 relative units (a positive cell cytotoxicity assay at 1:100 or 1:1000 dilution, respectively).
- This model has been used extensively to investigate the propensity of different antimicrobials to induce CDI and has been shown to be clinically reflective. Antibiotics known to have a high propensity to induce CDI clinically have induced simulated CDI in this model, as defined by detection of a proliferating vegetative cell population (increase of total viable counts vs spore counts) and detectable toxin production. Such examples include clindamycin,[1, 2] cephalosporins,[3, 4] co-amoxyclav[5] and fluoroquinolones including moxifloxacin.[6] However, antibiotics described as 'low-risk' for CDI clinically have not induced simulated CDI in the gut model (e.g. tigecycline,[7] piperacillin-tazobactam[8].
- This study provides data indicating that omadacyline may have a low risk for CDI induction, despite gut microbiota effects disrupting 'colonisation resistance'.
- Further *in vitro*, *in vivo* and human clinical data are required to confirm our data demonstrating low potential of omadacycline to induce CDI.

References

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