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

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Abstract

Background: Omadacycline is a potent aminomethycycline antibiotic with activity against Gram-positive (including MSSA/MRSA) and B. pneumoniae. Gram-negative, 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: Two stage chronic gut models were inoculated with a pooled human faecal slurry (n=6) from healthy volunteers (age 260 years) and left for 2 weeks to allow populations to equilibrate. The model was challenged with 10^5 cfu/mL C. difficile (strain NCTC027) on days 14 & 21. Omadacycline (43 mg/L) or moxifloxacin (43 mg/L) was instilled once daily, for 7 days from day 21. The model was observed for 3 weeks post-antimicrobial challenge (days 28-49). Gut microbiota populations and C. difficile total viable and spore counts were enumerated daily by culture on selective and non-selective agar. Toxin was detected by cell cytotoxicity assay, and antimicrobial concentrations were measured by bioassay.

Results: Gut microbiota populations were stable before antimicrobial challenge. Moxifloxacin instillation caused declines in B. fragilis group species (~4 log_10 cfu/mL), Enterococcus spp. (~4 log_10 cfu/mL) and Lactobacillus spp (~3 log_10 cfu/mL, in both V2 and V3). Concomitantly, simulated CDI and cytotoxicity production (3 relative units) was observed. Omadacycline instillation caused declines in populations of bifidobacteria (~1 log_10, cfu/mL), B. fragilis group species (~3 log_10, cfu/mL), Lactobacillus spp (~6 log_10 cfu/mL) and Enterococcus spp. (~4 log_10, cfu/mL), colistina (~1 log_10, cfu/mL) and Enterobacteriaceae (~5 log_10, cfu/mL). Despite these changes, no evidence of C. difficile germination, vegetative cell proliferation or toxin production were observed.

Conclusions: Omadacycline exposure caused marked disruption to gut microbiota populations. However, in contrast to exposure to moxifloxacin and many other antimicrobials (e.g. cindamycin, ceftriaxone, ofloxacin, and other fluoroquinolones) evaluated in the gut model, omadacycline promoted neither C. difficile proliferation nor simulated CDI. Human in vivo data are required to confirm this observed low potential of omadacycline to induce CDI.

Introduction

Omadacycline is an aminomethycycline antibiotic, currently in phase 3 clinical trials, with activity against a wide range of Gram-positive and Gram-negative bacteria, including MSSA/MRSA, MS or MR coagulase-negative staphylococci, Enterococcus faecalis, Enterococcus faecium, S. pneumoniae, Klebsiella pneumoniae, Proteus mirabilis/vulgatus, Providencia rettgeri/ Stuarti, Morganella morgani and Bacteroides fragilis. Its activity in vitro and in vivo has been extensively investigated. This model has been used to evaluate the potential of omadacycline to induce C. difficile infection (CDI) by using a well validated model of the human gut. The model is designed to reflect the normal gut microbiota and to assess the potential for induction of C. difficile infection.

The model has been validated against gut contents from human volunteers and provides a very close simulation of gut bacterial activity and composition in different areas of the hindgut. The model has been used to simulate C. difficile infection (CDI) using epidemic strains of C. difficile as well as to study the propensity of cefotaxime, cipempricillin-tazobactam, ertapenem, clindamycin or fluoroquinolones to induce CDI. This study examines the effects of omadacycline vs moxifloxacin, which has previously been shown to induce simulated CDI in the gut model, and has been clinically described as high risk for CDI.

Methods

Two models, consisting of three vessels aligned in series and top-fed with a complex growth medium, were assembled for this study. The models were inoculated with pooled human faeces from five healthy volunteers (age > 60 years) with no history of antibiotic therapy in the previous 3 months. All vessels were continuously stirred, anaerobically maintained at 37°C and regulated to reflect in vivo differences, including pH, from proximal to distal colon.

Results

- **Bacterial populations** were monitored in vessels 2 and 3, using viable counting on selective agars. Monitoring was performed every other day for the first 14 days and daily thereafter.
- **C. difficile** total viable counts and spores (following alcohol shock) were enumerated on Brauer's COEYL agar in Vessels 1, 2 and 3.
- From day 14 onwards, C. difficile cytotoxicity was assessed using a quantitative VERO cell cytotoxicity assay. Gut model supernatant was serially diluted 1:10 in sterile PBS to 10^6 and added to vero cell monolayers together with C. sordelli/antitoxin. Monolayers were examined after 24 and 48h incubation in 5% CO₂ with a positive result reported in the highest dilution with >70% cell rounding. Cytotoxic titres (relative units, RU) were an arbitrary log unit scale, i.e. 1RU=1000, 10RU=1000, 100RU=10000.
- **Bioassay:** From day 21 onwards, active concentrations of moxifloxacin were determined using tosonecist test agar with Escherichia coli as the indicator organism. Concentrations of omadacycline were determined using Wilkins Chalgren agar with Kocuria rhizophila as the indicator organism.

Conclusion

- The model used in this study has been shown to be clinically reflective. Antibiotics known to have a high propensity to induce CDI clinically have induced CDI in this model (e.g. cephalosporins, clindamycin, c-amoxycillin, and fluoroquinolones), whereas antibiotics considered as 'low-risk' for CDI clinically have not induced simulated CDI in the gut model (e.g. ligeicycline, piperacillin-tazobactam).
- Moxifloxacin instillation induced simulated CDI in the gut model in this study. Moxifloxacin instillation decreased *B. fragilis* group species (3 log_10 cfu/mL decline), Enterococcus spp. (3 log_10 cfu/mL decline) and Lactobacillus spp. (2 log_10 cfu/mL decline) populations. This disruption of gut microbiota populations was followed by C. difficile spore germination, vegetative cell proliferation and detectable toxin in all three vessels (V1 and V2 data not shown).
- Despite causing extensive disruption to the gut microbiota 'colonisation resistance', omadacycline exposure did not induce any signs of simulated CDI within the in vitro human gut model. The high intrinsic activity of omadacycline against C. difficile presumably prevents its expansion even when a potential niche has been created by antibiotic exposure.
- This study provides data indicating that omadacycline may be lower-risk for CDI induction. Further clinical evaluation is required to confirm this hypothesis.

References