Effectiveness of a short (4 day) course of oritavancin in the treatment of simulated Clostridium difficile infection using a human gut model

C. H. Chilton1, J. Freeman2, G. S. Crowther1, S. L. Todhunter1 and M. H. Wilcox1,2*

1Leeds Institute for Molecular Medicine, University of Leeds, Leeds LS2 9JT, UK; 2Department of Microbiology, Leeds Teaching Hospitals NHS Trust, The General Infirmary, Old Medical School, Leeds LS1 3EX, UK
*Corresponding author. Microbiology, The General Infirmary, Old Medical School, Leeds LS1 3EX, UK. Tel: +44-113-3926818; Fax: +44-113-3922696; E-mail: mark.wilcox@leedsth.nhs.uk

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Objectives: We previously demonstrated that 7 days of oritavancin instillation effectively treats Clostridium difficile infection (CDI) in a human gut model. Oritavancin may be more effective than vancomycin due to apparently increased activity against spores. We compared the efficacy of shortened dosing duration (4 days) of oritavancin and vancomycin for CDI treatment using the gut model.

Methods: Clindamycin induced CDI in two triple-stage chemostat gut models primed with pooled human faeces and C. difficile ribotype 027 spores. Oritavancin (64 mg/L twice daily) or vancomycin (125 mg/L four times daily) was instilled for 4 days and the effects on C. difficile proliferation and toxin production, and gut microflora were determined.

Results: Both oritavancin and vancomycin reduced toxin to undetectable levels. Recurrent C. difficile germination occurred 20 days after vancomycin instillation, with high-level toxin production. Oritavancin reduced C. difficile counts to around the detection limit for the remainder of the experiment, with spores undetectable from day 1 of instillation. Toxin production was reduced to below detectable levels, but was sporadically seen later, despite no evidence of germination. Both oritavancin and vancomycin instillation led to only modest effects on gut microflora.

Conclusions: Shortened courses of oritavancin and vancomycin effectively treated CDI in a human gut model, but evidence of recurrence was observed following vancomycin instillation. Oritavancin exposure inhibited the recovery of C. difficile spores, as previously described. Shortened antibiotic exposure minimizes disruption to the gut microflora. These data indicate the possible value of a 4 day oritavancin dosing regimen for CDI treatment.

Keywords: spores, gut microflora, ribotype 027

Introduction

Clostridium difficile infection (CDI) is a significant cause of morbidity, particularly in elderly hospitalized patients, and a major burden on healthcare institutions.1,2 CDI treatments remain limited (primarily oral metronidazole, vancomycin or fidaxomicin, typically for a minimum of 10 days).3,4 Recent reports of reduced metronidazole efficacy, notably in severe CDI cases, and reduced metronidazole susceptibility among epidemic C. difficile ribotypes,5,6 provide impetus to evaluate new antimicrobial agents. We previously described an in vitro human gut model of CDI that yields results consistent with clinical and animal model data,5,6 and have used this model to compare the efficacy of oritavancin (a novel potential CDI treatment compound) and vancomycin instilled for a 7 day period. Oritavancin effectively treated simulated CDI within the gut model, and may be superior to vancomycin due to apparent increased activity against spores.7 Recurrence of CDI is particularly problematic,7 and may be linked to the level of disruption of the gut microflora and C. difficile spore persistence. Thus, shorter CDI treatment duration, potentially associated with reduced disturbance of gut microflora, may be beneficial. Here we describe the efficacy of a shortened course (4 days) of either oritavancin or vancomycin for the treatment of simulated CDI in a human gut model.

Materials and methods

We have previously demonstrated that the human gut model is a clinically reflective method of evaluating potential treatments for CDI.6,11 The gut models were prepared, inoculated with faecal slurry and operated as previously described.6,11 Each gut model consisted of three vessels in a
weir cascade system, anaerobically maintained and operated at increasing pH (5.5, 6.2 and 6.8) to reflect the increasing alkalinity of the gut. Gut models were maintained at 37°C and top-fed with growth media. Models were primed with 10% faecal slurry prepared from pooled, C. difficile culture-negative faeces from healthy elderly volunteers (no antimicrobial therapy within 3 months).

After preparation and inoculation of the model with faecal slurry, the models were left to equilibrate in respect of bacterial populations, until a steady state was achieved (period A). Gut bacterial populations were monitored every other day, as previously described.6 The models were then inoculated with 107 cfu of C. difficile ribotype 027 spores (epidemic outbreak strain kindly supplied by Dr Rob Owens, Maine Medical Centre, Portland, USA) and monitored with no interventions (period B). Bacterial populations, C. difficile vegetative cell and spore counts, and cytotoxin titre were quantified daily thereafter. After 7 days a further inoculum of 107 cfu of C. difficile ribotype 027 spores was administered, and clindamycin dosing commenced (33.9 mg/L four times daily) for 7 days (period C). Following clindamycin dosing, no further interventions were made (period D) until C. difficile germination and high-level toxin production were observed. At this point, vancomycin (125 mg/L four times daily for 4 days) or oritavancin (64 mg/L twice daily for 4 days) instillation commenced (period E). No further interventions were made thereafter, and populations continued to be quantified daily for 21 days (period F).

Indigenous gut microflora organisms and C. difficile total viable counts (TVCs) and spore populations (log10 cfu/mL) were quantified by counting viable colonies on selective and non-selective agar. The following groups were quantified as previously described:6 total facultative aerobes (nutrient agar), lactose fermenters (MacConkey agar no. 3), enterococci (kanamycin aesculin azide agar), total anaerobes (fastidious aerobes (nutrient agar), lactobacilli (LAMVA8 agar), bifidobacteria (Beersens agar) and C. difficile TVCs and spores [Braziers cyclosorine cefoxitin egg-yolk agar with lysosome (CCEYL)]. C. difficile cytotoxin titres [relative units (RU)] were determined using a cell cytotoxin assay. Limits of detection (LODs) of these quantification methods are 1.22 log10 cfu/mL for microflora populations and C. difficile TVCs, 1.52 log10 cfu/mL for C. difficile spores and 1 RU for cytotoxin levels.

Antimicrobial concentrations were determined using an in-house, large-plate bioassay as described previously.7–9

## Results

The conditions within vessel 3 are the most similar to distal colon conditions in vivo, so quantitative microbiology results and toxin titres from this vessel are presented here. Clindamycin instillation elicited a decline in bifidobacteria (~7 log10 cfu/mL) and B. fragilis group (~2–5 log10 cfu/mL) populations, and an increase in enterococcal populations (~3–4 log10 cfu/mL) (data not shown). Similar changes in microflora populations within the gut model post-clindamycin-exposure have previously been described.7–9 In addition, clindamycin induced C. difficile germination and high-level toxin production as previously described.7–9 Oritavancin and vancomycin instillation commenced 11 and 14 days after germination occurred (following 7 and 2 days of toxin titres ≥3 RU, respectively).

C. difficile TVCs decreased immediately on oritavancin and vancomycin exposure, reaching the LOD 2 and 5 days after instillation commenced, respectively, and remaining around the LOD for most of the remainder of the experiment (Figures 1 and 2, periods E and F). Spore counts were around the LOD before oritavancin instillation, and were undetectable for the remainder of the oritavancin experiment (Figure 1, periods E and F). Vancomycin instillation reduced spore counts by ~2 log10 cfu/mL to around the LOD, but spores were detected sporadically thereafter (Figure 2, periods E and F). Oritavancin reduced toxin to undetectable levels by day 4 of treatment, in comparison with 8 days post-cessation of vancomycin dosing. Cytotoxin was detectable sporadically at low levels (1–2 RU) from 8 days after cessation of oritavancin treatment until the end of the experiment, but was not accompanied by germination/vegetative growth. C. difficile TVCs and spore counts remained around the LOD until 19 days after cessation of vancomycin instillation (period F), whereupon germination recurred. Vegetative counts increased to ~6 log10 cfu/mL accompanied by evidence of cytotoxin production (2 RU).

Oritavancin and vancomycin instillation had only modest effects on the gut microflora. The greatest effect was that of oritavancin on enterococcal counts, which decreased by

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**Figure 1.** Oritavancin treatment. Mean C. difficile PCR ribotype 027 TVCs and spore counts (log10 cfu/mL), cytotoxin titres (RU) and concentrations of clindamycin (CLI) and oritavancin (ORV) (mg/L) in vessel 3 of the oritavancin-treated gut model. Refer to main text for details of periods A–F.
Bacteroides populations returned to steady-state levels after cessation of clindamycin instillation, and no further substantial adverse affects on B. fragilis group counts were observed on exposure to oritavancin or vancomycin (Table 1).

Discussion

The human gut model has been used successfully for the evaluation of both propensity of antibiotics to induce CDI and efficacy of novel therapies for CDI. We have previously shown that oritavancin therapy may be more effective in treating CDI than vancomycin, the current generally preferred option, possibly due to activity against C. difficile spores. Notably, we and others have shown that 7–10 days vancomycin treatment of CDI is associated with inhibition of Bacteroides spp., presumably due to persistent very high luminal antibiotic concentrations. In order to minimize deleterious effects on gut flora, we investigated shortened (4 day) vancomycin and oritavancin dosing periods.

Both vancomycin and oritavancin successfully reduced C. difficile vegetative counts to around the LOD by the end of the 4 day instillation period (period E), while having minimal effects on the gut microflora. Oritavancin was more effective than vancomycin at reducing toxin levels (undetectable by 4 and 12 days after treatment commenced, respectively). Spores were also more effectively suppressed by oritavancin, as counts were at the LOD in the vancomycin-treated model, but spores were undetectable from day 1 of oritavancin instillation onwards. This indicates that the anti-spore effects of oritavancin, previously observed over a 7 day instillation regimen, were still evident despite a shortened course of treatment. Recurrence of symptomatic CDI may be associated with persistence or re-acquisition of spores. Spore recrudescence, germination and toxin production were observed following 4 days of exposure to vancomycin, but not after oritavancin. Spore recrudescence was also observed in the previously published 7 day vancomycin treatment gut model. Low toxin levels were observed following oritavancin treatment, but were not accompanied by evidence of

Table 1. Changes in bacterial population on instillation of 4 days of oritavancin or vancomycin, compared with the current standard treatment of 7 days of vancomycin

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>4 days of oritavancin</th>
<th>7 days of vancomycin</th>
<th>4 days of vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. difficile TVCs</td>
<td>5, decrease to around LOD</td>
<td>2, decrease</td>
<td>6, decrease to LOD</td>
</tr>
<tr>
<td>C. difficile spores</td>
<td>2, decrease to below LOD</td>
<td>1, decrease</td>
<td>2, decrease to around LOD</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>2, decrease, recovery</td>
<td>no change</td>
<td>no change</td>
</tr>
<tr>
<td>Total clostridia</td>
<td>3, decrease, recovery</td>
<td>4, decrease, recovery</td>
<td>no change</td>
</tr>
<tr>
<td>B. fragilis group</td>
<td>2, increase, recovery</td>
<td>7, decrease, recovery</td>
<td>2, decrease, recovery</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>no change, below LOD</td>
<td>2, decrease to LOD</td>
<td>no change, below LOD</td>
</tr>
<tr>
<td>Total facultative anaerobes</td>
<td>1, decrease</td>
<td>no change</td>
<td>no change</td>
</tr>
<tr>
<td>Lactose fermenters</td>
<td>2, decrease, recovery</td>
<td>1, decrease, recovery</td>
<td>3, decrease, recovery</td>
</tr>
<tr>
<td>Enterococci</td>
<td>6, decrease</td>
<td>2, decrease</td>
<td>2, decrease, recovery</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>no change</td>
<td>1, increase</td>
<td>no change</td>
</tr>
</tbody>
</table>

Recovery = return to pre-instillation levels.
germination. This may be due to toxin accumulation within the gut model biofilm and subsequent release back into the planktonic fluid (data not shown). However, lack of germination indicates that a 4 day oritavancin dosing regimen is superior to both 4 days and 7 days of vancomycin instillation\textsuperscript{7} in prevention of \textit{C. difficile} recrudescence in the gut model.

The use of a shortened dosing period, and consequently shorter duration of antimicrobial exposure, prevented the decline in \textit{Bacteriodes} spp. seen previously for 7 day vancomycin instillation,\textsuperscript{7} and other effects of 4 day antibiotic instillation on the gut microflora were minimal, although a decline in enterococcal viable counts was still observed on oritavancin exposure. Four days of oritavancin instillation was comparable to 7 days of oritavancin instillation in terms of effects on gut microflora populations (Table 1).\textsuperscript{7} The combination of minimal impact on the gut flora, while retaining the desirable anti-spore effects of oritavancin, and superiority over vancomycin in terms of spore recrudescence, indicates the possible value of a 4 day oritavancin dosing regimen in treatment of CDI.

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\section*{References}