Oritavancin does not induce Clostridium difficile germination and toxin production in hamsters or a human gut model

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Objectives: To evaluate the relative propensities of oritavancin and vancomycin to induce Clostridium difficile infection (CDI) in hamster and in vitro human gut models.

Methods: Hamsters received clindamycin (100 mg/kg orally or subcutaneously), oritavancin (50 mg/kg orally) or vancomycin (50 mg/kg orally). C. difficile spores were administered orally the next day. Control hamsters received vehicle only (polyethylene glycol 400) plus spores or clindamycin but no spores. Hamsters were monitored for clinical signs for 20 days. Caecal contents were analysed for C. difficile cells, spores and the presence of (cyto)toxin. Oritavancin and vancomycin were instilled over 7 days into separate in vitro gut models primed with pooled human faeces and inoculated with C. difficile ribotype 027 spores. Gut flora, C. difficile total viable and spore counts, toxin titres and antimicrobial concentrations were determined.

Results: All hamsters treated with oritavancin survived up to 20 days, with no evidence of C. difficile spores, vegetative cells or toxin in their caeca. No hamsters treated with clindamycin or vancomycin survived >6 days after spore administration. Death was associated with high C. difficile counts and toxin in caecal contents. In the gut model, oritavancin dosing elicited a rapid, marked decrease in total viable C. difficile and spore counts to below the limit of detection. Vancomycin did not elicit germination or toxin production in the gut model, but C. difficile remained present as spores throughout.

Conclusions: Oritavancin exposure, unlike exposure to vancomycin or clindamycin, did not lead to CDI in hamsters. In both models, oritavancin reduced C. difficile total counts and spores to below detectable limits. The data indicate the potential of oritavancin for CDI treatment, since exposure did not induce C. difficile germination and toxin production, which are known to exacerbate the disease state.

Keywords: spore inhibition, ribotype 027, microflora

Introduction

Clostridium difficile infection (CDI) is a major healthcare burden and a significant cause of morbidity in the hospitalized elderly. A recent systematic review of studies reported associated costs of up to US$4846 and US$8570 per primary CDI case (in US and non-US investigations, respectively, with further increases for recurrent cases). Increases in CDI incidence and poor outcome have been associated with the emergence of C. difficile PCR ribotype 027 (NAP 1). While certain antimicrobials represent a markedly increased risk of CDI, almost every class of antimicrobial has been implicated in CDI.

Conventional antimicrobial treatment for CDI is limited to either metronidazole or vancomycin, with fidaxomicin being approved in 2011 in the USA and Europe to treat C. difficile-associated diarrhoea. Metronidazole was, until recently, the treatment of choice for reasons of cost and possibly reduced likelihood of vancomycin-resistant enterococci (VRE) selection. However, recent reports of reduced metronidazole efficacy, notably in severe CDI cases, and reduced metronidazole susceptibility among epidemic CD ribotypes has seen a reduction in its use in favour of vancomycin. Notably, both metronidazole and vancomycin are associated with high rates of CDI recurrence, which is thought to be due to either reacquisition or persistence of C. difficile spores. Fidaxomicin is associated with a reduced risk of recurrent CDI, but the evaluation of novel treatments for CDI is still clearly warranted.

Oritavancin is a lipoglycopeptide that shows in vitro activity against C. difficile. O’Connor et al. demonstrated similar activity to vancomycin by agar incorporation (geometric mean 1.58...
against a panel of genotypically distinct and clonal strains, including some with reduced metronidazole susceptibility. However, this study found that oritavancin activity is likely to be underestimated by agar incorporation methods. We have previously demonstrated that oritavancin is highly effective in an in vitro gut model of CDI, whilst sparing gut microflora.9 Furthermore, oritavancin, unlike vancomycin, inhibited C. difficile spore outgrowth in a human in vitro gut model of CDI. In addition, experiments in the hamster model demonstrated that oritavancin protects hamsters from CDI for longer than vancomycin.10 We have now investigated the relative propensities of oritavancin and vancomycin to induce CDI in both an in vitro gut model and the hamster model.

Methods

In vitro gut model of CDI

The gut model was designed by MacFarlane et al.11 and validated against the gut contents of sudden death victims. We have described its modification and use for the evaluation of antimicrobials as potential CDI treatments, or for their propensity to cause CDI.9,12–16 Briefly, the model consists of three fermentation vessels arranged in a weir cascade system and top-fed with growth medium at a controlled rate (dilution rate $D=0.015/h$). The system is sparged with oxygen-free nitrogen, to maintain anaerobiosis and is maintained at 37°C via a circulating water jacket system. Each vessel is pH regulated to reflect the increasing alkalinity of the gut from proximal to distal. Vessel 1 is maintained at pH 5.5, vessel 2 at pH 6.2 and vessel 3 at pH 6.8. The gut model is inoculated with a 10% (w/v) slurry and left to equilibrate in respect of bacterial populations for 14 days.

Preparation of gut model

Faecal samples were collected from five healthy elderly (>65 years) volunteers with no history of antimicrobial treatment for 2 months prior to donation. Faeces were screened for the presence of C. difficile using modified Brazier’s CCEYL agar. C. difficile culture-negative faeces were pooled and a coarse-filtered 10% (w/v) slurry was prepared in pre-reduced PBS. The gut model vessels were then filled to two-thirds volume and the media pumps started.

Bacterial enumeration

Major culturable components of the gut microflora and C. difficile in vessels 2 and 3 were enumerated using selective and non-selective agars as described previously.17 Bacterial groups isolated and enumerated were total facultative aerobes, lactose fermenters, enterococci, total obligate anaerobes, lactobacilli, bifidobacteria, Bacteroides fragilis group, total clostridia, C. difficile total viable counts [limit of detection (LOD) $= 1.22 \log_{10}$ cfu/mL] and spore counts [LOD $= 1.52 \log_{10}$ cfu/mL]. C. difficile toxin was quantified using a Vero cell cytotoxicity assay as previously described;18 cytotoxin titres were expressed in log$_{10}$-relative units (RU) [LOD $= 1.1$ RU]. In vessel 1 of the gut model, only C. difficile total bacterial counts, spore counts and cytotoxin titres were enumerated.

Experimental design

Two gut models were inoculated with faecal slurry and allowed to equilibrate in respect of bacterial populations for 14 days, with no interventions (period A). Bacterial populations were monitored every other day during this period. At this point (day 14) a single inoculum of $\approx 10^7$ cfu C. difficile PCR ribotype 027 spores was introduced into vessel 1 of each gut model with no further interventions for 7 days (period B). Bacterial populations were monitored every day during this period and thereafter. On day 21, a further inoculum of $\approx 10^7$ cfu C. difficile PCR ribotype 027 spores was introduced into vessel 1 of each gut model. Gut models were then instilled with either oritavancin (64 mg/L, twice daily for 7 days) or vancomycin (125 mg/L, four times daily for 7 days) (period C). Following cessation of antibiotic instillation, no further interventions were made and bacterial populations were monitored for 21 days (period D). This experimental design differs from our previously described oritavancin gut model,9 as it does not contain a clindamycin instillation period to induce simulated CDI. The study therefore examines the propensity of oritavancin to induce CDI within a human gut model, as opposed to the effectiveness of oritavancin as a treatment for simulated CDI.

Bioassay of antimicrobial concentrations

Vancomycin and oritavancin concentrations within the vessels of each gut model were determined using an in-house large plate bioassay as previously described.9 The vancomycin bioassay used Staphylococcus aureus ATCC 29213 as the indicator organism, while the oritavancin bioassay used Kocuria rhizophila ATCC 9341. Vancomycin calibrators were prepared in sterile deionized water. Oritavancin calibrators were prepared in deionized water containing 0.002% polysorbate 80. Indicator organism (1 mL; turbidity equivalent to that of a 0.5 McFarland standard) was added to cooled, molten Mueller–Hinton agar supplemented with 1 M para- amino benzoic acid. Twenty-five 9-mm-diameter wells were removed from the agar using a number 5 cork borer. Samples from the gut model were centrifuged (16000g) and sterilized by filtration (0.22 µm). Oritavancin gut model samples and calibrators were not filtered, since the drug binds to cellulose acetate filters. Twenty microlitres of each doubling dilution of vancomycin calibrator (1–512 mg/L), oritavancin calibrator (1–128 mg/L), or sample from the gut model was assigned randomly to each well in triplicate. Agar plates were refrigerated (4°C) for 5 h to allow antimicrobial diffusion whilst minimizing bacterial growth. Vancomycin bioassay plates were then incubated aerobically at 37°C for 48 h, while oritavancin bioassay plates were incubated anaerobically at 30°C for 48 h. Zone diameters were measured using digital callipers (Fisher Scientific) accurate to 0.2 mm and calibration lines produced by plotting diameter squared against log$_2$-concentration of antimicrobial. Unknown antimicrobial concentrations were read from the calibration line for each plate and converted to actual concentrations using an inverse log$_2$ function. Mean antimicrobial concentrations (mg/L) were averaged from the three replicates. Limits of detection (LODs) for the oritavancin and vancomycin bioassays were 2 and 8 mg/L, respectively.

Hamster model of CDI

Animal care and use practices in this study were in accordance with the Guidelines and Policies of the American Association of Laboratory Animal Care, International and the Canadian Council on Animal Care. All the experimental protocols were approved by the local Animal Care Committee (Mispro Biotech Services Inc.). Mispro Biotech Services is a contract research services company (Montreal, Canada), is independent of The Medicines Company, and is accredited by both the Canadian Council on Animal Care and the Association for Assessment and Accreditation of Laboratory Animal Care International. Animals had free access to food and water throughout the experiment. For identification and temperature monitoring, a subcutaneous microchip was implanted in each hamster (Bio-Medic system). Hamsters were housed two or three per cage and cages were changed for 4 consecutive days between clindamycin administrations (day $-1$) up to 2 days after spore administration. One day prior to infection (day $-1$), vehicle alone (85% PEG 400 in water, $n=10$),
clindamycin (100 mg/kg, n = 10), vancomycin (50 mg/kg, n = 22) formulated in 85% PEG 400 or oritavancin (50 mg/kg, n = 22), also formulated in 85% PEG 400, was administered orally to male Syrian golden hamsters (65–80 g) in the absence of any other pretreatment. One day later (day 0), animals were infected orally with \( \sim 10^3 \) spores of \( \textit{C. difficile} \) CD9925 (PCR ribotype 027) (a gift from Dr Jacques Pepin, Universite´ de Sherbrooke, Canada), which had been prepared by ethanol treatment of \( \textit{C. difficile} \) cells incubated anaerobically for 7 days at 37°C. One group of animals (n = 10) that had received clindamycin pretreatment received no spores. The following observed clinical signs reflective of \( \textit{C. difficile} \) infection were monitored: wet tail, severe diarrhoea, reduction in core body temperature of 4°C, reduced activity.

**Detection of \( \textit{C. difficile} \) in caecal contents**

The caecal contents of animals at both clinical and experimental endpoints were screened for \( \textit{C. difficile} \) cells and toxins. The presence of \( \textit{C. difficile} \) toxins (toxin A and B) was assessed using the CD TOX A/B ITIM kit (Techlab) as described by the manufacturer. The LODs were \( \sim 0.8 \) ng/mL for toxin A and \( \sim 2.5 \) ng/mL for toxin B. Total viable cells (both vegetative and spore forms) were enumerated after 10-fold serial dilutions in PBS containing 2% Oxystre (Oxystre Inc.) and plating on modified Brazier’s CCEYL agar. \( \textit{C. difficile} \) spores were counted by treating caecal samples with an equal volume of pure ethanol followed by plating of the resulting suspension. The LODs were 1.47 and 1.77 log_{10} cfu/g caecal content for total viable counts and spores, respectively.

**Results**

**In vitro gut model experiments**

In both gut models, intestinal microflora components were unaffected by the administration of \( \textit{C. difficile} \) spores, maintaining their steady-state levels. \( \textit{C. difficile} \) spore numbers steadily declined at approximately the rate of dilution.

**Effect of oritavancin on \( \textit{C. difficile} \)**

Following commencement of oritavancin instillation, \( \textit{C. difficile} \) was undetectable in all vessels either as total viable counts or spores by day 2 of oritavancin dosing. This represents a decrease of \( \sim 5 \) log cfu/mL. \( \textit{C. difficile} \) was not isolated from vessel 1 of the gut model until 7 days after cessation of antibiotic dosing. Therefore, spores were isolated intermittently from this vessel at around the LOD (data not shown). \( \textit{C. difficile} \) spores were isolated from vessel 2 of the gut model on only one occasion (day 46) and at around the LOD. \( \textit{C. difficile} \) was not isolated from vessel 3 of the gut model after dosing (Figure 2a). Cytotoxin was not detected in any vessel throughout the experiment.

**Effect of vancomycin on \( \textit{C. difficile} \)**

\( \textit{C. difficile} \) remained as spores in all vessels during vancomycin dosing and thereafter. \( \textit{C. difficile} \) spores continued to be eliminated from all vessels at approximately the rate of dilution (Figure 2b). In vessel 2, \( \textit{C. difficile} \) spore counts and total viable counts diverged briefly at day 39, suggesting germination, but counts reconverted at around the LOD 4 days later. Cytotoxin was not detected in any vessel throughout the experiment.

**Antimicrobial concentrations in the gut model**

Oritavancin concentrations peaked at 128, 419 and 593 mg/L in vessels 1, 2 and 3, respectively, and were detectable for 16, 20 and 27 days, respectively, after commencement of instillation (vessel 3 concentrations shown in Figure 2a). Vancomycin concentrations peaked at 660, 592 and 485 mg/L in vessels 1, 2 and 3, and were detectable for 12, 13 and 16 days after commencement, respectively (vessel 3 concentrations shown in Figure 2b).

**Hamster model experiments**

Hamsters that were not exposed to \( \textit{C. difficile} \) spores (n = 10) on day 0 showed 100% survival at day 20, the experimental endpoint (Figure 3). Likewise, those exposed to \( \textit{C. difficile} \) spores, but given no antimicrobial pretreatment on day −1 (n = 10), also showed 100% survival. Neither \( \textit{C. difficile} \) spores nor vegetative cells were recovered from caecal contents, nor was there any evidence of \( \textit{C. difficile} \) toxin (Table 1).

All hamsters that had been pretreated with 100 mg/kg clindamycin (n = 10) or 50 mg/kg vancomycin (n = 22) died by day 6 and day 5 post infection, respectively (Figure 3). Both showed high levels of \( \textit{C. difficile} \) spores (mean 6.73 ± 0.31 and 6.14 ± 0.71 log cfu/g of caecum, respectively), and higher levels of \( \textit{C. difficile} \) vegetative forms (mean 10.1 ± 0.62 and 10.2 ± 0.33 log cfu/g caecum, respectively) (Table 1). \( \textit{C. difficile} \) toxin was demonstrable in the caecal contents of both clindamycin- and vancomycin-treated hamsters.

In contrast, all hamsters pretreated with 50 mg/kg oritavancin (n = 22) survived to day 20. \( \textit{C. difficile} \) spores, vegetative cells and toxin were not detected in caeca from oritavancin-pretreated hamsters (Table 1).
Discussion

The present study used hamster and *in vitro* gut models to assess the likelihood of antibiotic predisposition to CDI. We compared vancomycin and oritavancin, established and investigational treatment options for CDI, with clindamycin. Thus, we have determined the effect of antibiotic exposure on gut contents, and in turn the capacity of *C. difficile* to multiply and produce toxins (or persist) as occurs in CDI. Results in the two models were consistent, as has been reported previously.12

There was no evidence of *C. difficile* germination and toxin production following oritavancin exposure in either the hamster or gut models. We have previously reported the use of an *in vitro* gut model for the comparative evaluation of oritavancin versus vancomycin treatment following clindamycin-induced *C. difficile* germination and toxin production.9 We found that both oritavancin and vancomycin effectively eliminated *C. difficile* vegetative cells from the gut model, but that oritavancin demonstrated potential activity against spores. We were unable to recover *C. difficile* spores from the gut model other than as sporadic single colonies.9 Similar observations were made in the present studies, in both the gut model and the hamster model. In the gut model, *C. difficile* vegetative and spore counts were reduced to below the LOD by day 2 of oritavancin instillation.

![Figure 1. Mean (±SEM) viable counts (log_{10} cfu/mL) of culturable indigenous gut microflora in vessel 3 of (a) oritavancin and (b) vancomycin gut models. Vertical lines indicate the final day of each experimental period. For descriptions of periods A–D, please refer to the Methods section.](https://academic.oup.com/jac/article/67/12/2919/775804)
with spores isolated only once from vessel 2, and not at all from vessel 3. In contrast, there was no vancomycin-mediated reduction in spores, which were eluted from the gut model at the rate of dilution, despite the increased dose and frequency of instillation of vancomycin compared with oritavancin. Likewise, *C. difficile* spores and vegetative cells were detected in vancomycin-treated hamsters, which were also symptomatic. In contrast, oritavancin-treated hamsters remained healthy and had no detectable *C. difficile* or toxin. Oral administration of oritavancin to hamsters achieves high gut concentrations; daily doses of oritavancin of 10, 50 and 100 mg/kg were associated with a $C_{\text{max}}$ in caecal contents of between 196 and 1922 mg/kg, but very limited systemic absorption (<0.1% bioavailability).

These findings are consistent with data showing that oritavancin can inhibit *C. difficile* spores.\(^5,18\) We have also seen a similar effect on spores following ramoplanin treatment of clindamycin-induced CDI in the gut model.\(^12\) Oritavancin is functionally related to ramoplanin and the lantibiotic nisin, which has also been reported to inhibit both *Bacillus subtilis* and *Clostridium sporogenes* spore outgrowth.\(^19,20\) We previously demonstrated that oritavancin interacts with and disrupts the transition from a germinated *C. difficile* spore to vegetative cell to a greater

**Figure 2.** Mean (±SEM) *C. difficile* PCR ribotype 027 total counts and spore counts (log_{10} cfu/mL), cytotoxin titres (RU) and antimicrobial concentrations (mg/L) in (a) oritavancin and (b) vancomycin experiments in vessel 3 of the gut model. The horizontal broken line indicates the LOD for bacterial culture. For descriptions of periods B–D, please refer to the Methods section.
extent than existing therapeutic antimicrobials for CDI. Oritavancin adheres to plastic surfaces, probably mediated by electrostatic charge, and this can be reduced by the surfactant polysorbate 80. It is therefore possible that oritavancin adheres to the spore surface, preventing effective outgrowth because of early cell death once germination is under way. Oritavancin has been shown to have activity against slow-growing *S. aureus* biofilm cells in vitro; such effects could be due to its adhesion to surfaces and/or its disruption of membrane potential and increased cell permeability mechanisms. Whether oritavancin binding to bacterial cell surfaces and disruption of bacterial membrane integrity might account for its activity against vancin binding to bacterial cell surfaces and disruption of bacterio- and increased cell permeability mechanisms.

**Figure 3.** Comparison of oritavancin, clindamycin and vancomycin pretreatment in inducing lethal CDI in hamsters. Hamsters were pretreated orally with oritavancin (ORI; 50 mg/kg), clindamycin (CLI; 100 mg/kg) or vancomycin (VAN; 50 mg/kg) 1 day before infection (day 0). Spores were administered on day 0 and survival was monitored for 20 days.

![Comparison of oritavancin, clindamycin and vancomycin pretreatment in inducing lethal CDI in hamsters.](image)

**Table 1.** Recovery of *C. difficile* vegetative cells, spores and toxin from hamster caecal content according to pretreatment

<table>
<thead>
<tr>
<th>Pretreatment (day -1)</th>
<th>Spores administered (day 0)</th>
<th>Percentage survival&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean (SD) spore count (log&lt;sub&gt;10&lt;/sub&gt; cfu/g caecum)</th>
<th>Mean (SD) vegetative cell count (log&lt;sub&gt;10&lt;/sub&gt; cfu/g caecum)</th>
<th>Toxin A/B in caecum (positive/negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>-</td>
<td>100</td>
<td>BLOD</td>
<td>BLOD</td>
<td>negative</td>
</tr>
<tr>
<td>PEG 400 85%</td>
<td>+</td>
<td>100</td>
<td>BLOD</td>
<td>BLOD</td>
<td>negative</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>+</td>
<td>0</td>
<td>6.73 (0.31)</td>
<td>10.1 (0.62)</td>
<td>positive</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>+</td>
<td>0</td>
<td>6.14 (0.71)</td>
<td>10.2 (0.33)</td>
<td>positive</td>
</tr>
<tr>
<td>Oritavancin</td>
<td>+</td>
<td>100</td>
<td>BLOD</td>
<td>BLOD</td>
<td>negative</td>
</tr>
</tbody>
</table>

BLOD, below the LOD (1.47 and 1.77 log<sub>10</sub> cfu of spores or vegetative cells per gram of caecal content, respectively).

<sup>a</sup>At day 20.
Oritavancin concentrations differed considerably from those observed in our previously published study. Oritavancin concentrations in vessel 3 were up to 26 times greater than those previously seen on the equivalent dosing day. This may explain the modest differences in effects of oritavancin on gut microflora in the current study compared with previous gut model data, and also the complete absence of C. difficile spores in vessel 3. The accumulation of antibiotic is thought to have been due to a media pump malfunction, but this appeared to have had a relatively minimal impact upon C. difficile behaviour.

In general, gut flora components were affected to a largely similar degree by oritavancin and vancomycin, although there were some drug-specific effects. Both agents produced marked deleterious effects upon B. fragilis group bacteria, clostridia and enterococci. Bifidobacterial numbers were unaffected by oritavancin, but slightly decreased by vancomycin. Conversely, lactobacilli were unaffected by vancomycin, but in some instances increased by oritavancin exposure. Vessel 2 showed a ~2 log increase in lactobacilli populations on oritavancin instillation (data not shown), whereas lactobacilli populations in vessel 3 remained more stable. We observed a marked difference in the magnitude of B. fragilis group inhibition between vessel 2 (8 log cfu/mL) and vessel 3 (2 log cfu/mL) of the oritavancin-dosed gut model. This is difficult to explain given that there were much higher concentrations of oritavancin in vessel 3. However, the precipitous decline coincided with a spike in oritavancin concentrations, and subsequent falls in antibiotic concentration were accompanied by recovery of B. fragilis group counts. Occasional blockage and release of the weir cascade tubing can occur, and this may have led to a temporary accumulation of oritavancin in vessel 2.

Despite the lack of sustained germination in the vancomycin-treated gut model, the presence of C. difficile spores indicates the potential for recrudescence (i.e. C. difficile growth with subsequent toxin release). This is in contrast to the oritavancin-treated gut model, in which there was a very marked reduction in spores to below the LOD. Together with the data from the hamster model, there appears to be a lower risk of C. difficile recrudescence following oritavancin than vancomycin exposure, which could translate into a lower risk of CDI recurrence. Further clinical evaluation of the potential of oritavancin to be an effective treatment for CDI, and particularly to reduce recurrence, is warranted.

### References


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### Transparency declarations

J. F. has received financial support to attend meetings from Bayer and Wyeth. M. M. was a contractor of The Medicines Company and does not have stock options in the company. G. M. and D. L. are full-time employees of The Medicines Company and have stock options in the company. M. H. W. has received honoraria for consultancy work, financial support to attend meetings and research funding from Astellas, AstaZeneca, Bayer, bioMérieux, Cerexa, Novabira, Novacta, Pfizer, Summit, The Medicines Company and Viropharma. All other authors: none to declare.


