Fidaxomicin inhibits toxin production in Clostridium difficile

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Objectives: Fidaxomicin, which was recently approved for the treatment of Clostridium difficile-associated diarrhoea, demonstrates narrow-spectrum bactericidal activity via inhibition of RNA polymerase. In this study we evaluated its inhibitory activity versus C. difficile toxin gene expression and toxin production by quantifying toxin mRNA and protein.

Methods: The effects of fidaxomicin, its major metabolite (OP-1118), vancomycin and metronidazole on toxin A and toxin B production were determined by assaying culture supernatants of two C. difficile isolates (ATCC 43255, a high-level toxin-producing strain, and UK-14, a NAP1/027/BI epidemic strain) using a commercial ELISA. The effects of the drugs on toxin gene expression were assessed in stationary-phase cells of C. difficile strain UK-1 (NAP1/027/BI type epidemic strain) and in the closely related non-epidemic strain CD196 by quantitative RT–PCR.

Results: Subinhibitory levels (1/4 × MIC) of fidaxomicin or OP-1118 (but not vancomycin or metronidazole) strongly suppressed toxin production in C. difficile (≥60%) through at least 1 week of culture. Additionally, transcripts from the pathogenicity loci (tcdR, tcdA and tcdB) were nearly completely inhibited by both fidaxomicin (2 × MIC) and OP-1118 (2.5 × MIC), but not vancomycin (2.5 × MIC).

Conclusions: Both fidaxomicin and OP-1118 are able to inhibit toxin production in vitro, which may explain prior post-treatment observations of less frequent detectable toxin in fidaxomicin-treated patients (27 subjects) than those treated with vancomycin (8 patients).

Keywords: vancomycin, metronidazole, diarrhoea, antibiotics, MICs

Introduction

Infections associated with Clostridium difficile can range in severity from mild diarrhoea to severe life-threatening pseudomembranous colitis, with a significant annual economic burden that is estimated to exceed $3 billion within the US healthcare system.1,2 The most recent upsurges in C. difficile infection (CDI) have been attributed to the emergence of hypervirulent strains, including, but not limited to, the North American pulstype 1 (NAP1)/PCR-ribotype 027/restriction endonuclease analysis (REA) group BI.3,4 The apparent increase in virulence of these strains has been attributed to various factors, including antibiotic resistance, enhanced motility and adherence, efficient sporulation and/or germination, and production of different toxins (TcdA, TcdB and binary toxin [C. difficile transferase (CDT)]).5,6,7,8 While the role of binary toxin in CDI is not well defined, both TcdA and TcdB, which are large glucosyltransferases, are known to inactivate host GTPases and also to provoke inflammatory responses; both mechanisms contribute to CDI-associated tissue damage and disease symptoms.6 It has been suggested that higher production of TcdA and TcdB or a more potent form of TcdB may contribute to hypervirulence of the strains.3,7,9 Both TcdA and TcdB are typically released during stationary phase and this can be accelerated under conditions of environmental stress, including treatment with certain antibiotics.10–12

Fidaxomicin, which was recently approved in the USA, Canada and Europe for the treatment of CDI, is a narrow-spectrum antibiotic with a unique mechanism of action that inhibits bacterial transcription at a very early stage of transcriptional initiation.13,14 During a fidaxomicin clinical trial, Louie et al.15 compared C. difficile toxin levels post-treatment (11–18 days) in a small number of subjects treated with fidaxomicin (27) versus control patients who received vancomycin (8) and noted that toxin B was less frequently detected in fidaxomicin-treated subjects than in vancomycin-treated subjects (1/27 versus 3/8, P = 0.3).
In this study, we quantified the effect of fidaxomicin, its major metabolite (OP-1118) and comparator drugs on the expression of toxin genes and toxin proteins in *C. difficile* cells.

**Methods**

**Bacterial strains and antimicrobial agents**

*C. difficile* strain ATCC 43255 was from the American Type Culture Collection (Manassas, VA, USA) and strain UK-14 (an REA group B1 strain, isolated during an outbreak in England and referred to as Meridian Biosciences strain number SM8-6865) was kindly provided by Dr Dale Gerding (Hines VA, IL, USA). Both strains were stored at −70 °C in Brucella broth supplemented with 5 mg/L haemin and 1 mg/L vitamin K [supplemented Brucella broth (SBB)] containing 10% glycerol, and these were used to study the effect of drugs on toxin production at Optimer Pharmaceuticals, Inc. Strain UK-1 (a NAP1/027/BI strain, isolated from the Stoke Mandeville Hospital outbreak) was also obtained from Dr Dale Gerding. Strain CD196 (considered to be the closest non-epidemic ancestor of the 027 strains) was obtained from Dr Michel Popoff. The UK-1 and CD196 strains were stored at −80 °C in brain heart infusion (BHI) medium containing 15% glycerol and were used to study the effect of drugs on the expression of toxin genes at Tufts University School of Medicine.

Vancomycin and metronidazole (both obtained from Sigma–Aldrich) were prepared as 10 mg/mL stock solutions in water and methanol, respectively. Similar stock concentrations of fidaxomicin and OP-1118 were prepared by dissolving the compounds in DMSO. All drugs were diluted further to an appropriate concentration in growth medium before use for MIC determination and for their effects on toxin production and toxin gene expression.

**In vitro susceptibility testing**

MICs were determined under settings designed to match the planned conditions of the toxin production and messenger RNA (mRNA) expression experiments. The CLSI microbroth (rather than agar) dilution method with slight modification was used for MIC determination for the ATCC 43255 and UK-14 strains, as previously described.17,18 Lyzed blood, which obscures the colour of media, was omitted from the culture medium (without affecting the growth of *C. difficile*) so that the redox indicator resazurin could be used to monitor the anaerobicity of the chamber and medium. Briefly, microtitre plates with serially diluted drugs were equilibrated in an anaerobic glove box for a minimum of 3 h. *C. difficile* cells (10^5 cfu) were added to each well. Following a 48 h incubation at 35 °C, the plates were examined for growth. The MIC was defined as the drug concentration at which no growth or the most significant reduction in growth was observed.

To determine MICs for strains CD196 and UK-1, susceptibility tests were performed in BHIS broth [BHI medium (Difco) containing 0.5% yeast extract and 0.1% l-cysteine] as previously described.17 The MIC was defined as the lowest concentration of antibiotic that prevented visible turbidity after 18 h.

**Kinetics of *C. difficile* growth and toxin (TcdA and TcdB) production**

Overnight cultures of *C. difficile* strains ATCC 43255 and UK-14, grown on blood agar plates (Hardy Diagnostics, Santa Monica, CA, USA), were suspended in SBB medium to an OD_{600} ≈ 0.4. The suspension was further diluted in SBB (1:100) and incubated at 37 °C in an anaerobic chamber until the cells reached the early stationary phase of growth (~10 h for the UK-14 strain and ~24 h for the ATCC 43255 strain). Sub-MIC concentrations (1/4× and 1/8× MIC) of drugs were then added to the cells, and cells were monitored for a period of ~1 week. Samples were withdrawn at different timepoints for quantification of total cfu by plating serially diluted samples on BHIS agar supplemented with 0.1% taurocholate (BHIS-TA). Additionally, at each timepoint, culture supernatants were aliquoted and stored at −70 °C for toxin quantification at a later time.

Quantification of TcdA and TcdB was performed using a commercial ELISA method (tgcbiOMICS GmbH, Mainz, Germany) according to the manufacturer’s instructions. Briefly, microtitre plates coated with capture antibodies to both TcdA and TcdB were incubated with culture supernatants or standard control toxins (appropriately diluted and tested in duplicate) for 60 min at 37 °C. Following washing of the unbonded material, specific monoclonal antibodies to either TcdA or TcdB (conjugated to horseradish peroxidase) was added to wells; microtitre plates were incubated for 60 min at 37 °C. Subsequent to a second wash step, substrate was added to allow colour development at room temperature for 30 min. The reaction was stopped by addition of H_2SO_4 to each well, and the ELISA was analysed by a spectrophotometer at 450 and 620 nm utilizing BioTek Gen5 Version 2.0 Data Analysis Software (Winooski, VT, USA).

**Toxin quantification data analysis**

A standard curve for generating data with Gen5 software was achieved using in-house prepared supernantant from *C. difficile* ATCC 43255, which was calibrated to the purified standards supplied by tgcbiOMICS. The in-house standard was prepared by growing *C. difficile* ATCC 43255 in SBB at 35 °C for 72 h. Following centrifugation to remove the cells, aliquots of the supernatant were stored at −70 °C and used in each run.

Concentrations generated using Gen5 software were corrected based on the dilutions of the supernatants used in the ELISA runs. Final average concentrations, for each duplicate drug-treatment sample, were normalized versus the average concentration values of the no-drug control to obtain the percentage of toxin present at each timepoint. In situations where one of the duplicate values was outside the limit of quantification, only the value within the limits was used for normalization. When both values for the least-diluted sample were below the lower limit of quantification (LLOQ), the value was imputed as the LLOQ for normalization and presentation. Experimental runs where most sample data points were near or below the LLOQ were rejected, and those runs were not used for the analysis of toxins present.

**Quantitative RT–PCR analysis of *C. difficile* toxin gene expression**

Overnight cultures of *C. difficile* grown in tryptose/yeast extract (TY) medium were used to inoculate fresh TY medium at OD_{600} ~0.05. Drugs (fidaxomicin at 2× MIC, OP-1118 at 2.5× MIC or vancomycin at 2.5× MIC) were added to the cultures either at the onset of stationary phase (T_0) or 2 h after T_0 (T_2). RNA was prepared from bacterial cells harvested at T_0, T_2 and T_4 (4 h after T_0) as previously described.19,20 RNA was quantified by absorbance using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). Primers for quantitative PCR (qPCR) were designed using the online PrimerQuest tool from Integrated DNA Technologies (http://www.idtdna.com/Scitools/Applications/Primerquest) and amplification efficiencies for each primer set were determined prior to use. Synthesis of cDNA was performed on 500 ng of RNA using random hexamer primers and the QuantiTect® Reverse Transcription Kit (Qiagen) according to the manufacturer’s recommendations. To control for chromosomal DNA contamination, mock cDNA synthesis reactions containing no reverse transcriptase were used as negative controls in subsequent amplifications. cDNA samples were diluted 4-fold and used as templates for qPCR of rpoC (primers oLB122 (CTAGCTGCCTCTGATGCTCA0C) and oLB123 (CCAGTCCTCTGATGCTCA0C), 16S rRNA (primers oLB189 (GATT-TACTCGGTAAGACGCG) and oLB190 (CTTACAAACTAGCTTATGCAGACG)),
tcdR [primers oLB143 (ACTCAGTAGATGATTGCAAGAAA) and oLB144 (CTGTTTCTCCCTCTCCATAATGT)], tcdA [primers oLB131 (GTATGGATGTTGGAGAAGTCA) and oLB132 (CTCTTCCTCTAGTAGCTGTAATGC)], tcdB [primers oLB141 (GGCAATATGTTACGCTACTCA) and oLB142 (TCGACTACAGTATTCTCTGAC)] and tcdC [primers oLB145 (CACAAAGGGTGCTACTG) and oLB146 (GTTAGAAATGACCTCCTCATGGTC)] using Roche SYBR Green 1 PCR mix and a Roche LightCycler 480 II thermocycler. Reactions were performed in a final volume of 20 μL using 4 μL of diluted cDNA and primers at 1 μM final concentration. Amplification included 45 cycles of the following steps: 10 s at 95°C, 10 s at 53°C and 15 s at 72°C. Reactions were performed in triplicate using cDNA synthesized from each of a minimum of two biological replicates, and results are presented as the means and SEM of the data obtained. Results were calculated using the 2^(-ΔΔCt) method, in which the amount of target mRNA is normalized to that of an internal control transcript (rpoC or 16S rRNA).

Results

Susceptibility testing

To choose drug levels for use during kinetics and gene expression studies, drug MIC values were first determined via a microbroth dilution method with inclusion of the CLSI quality-control strain. Reproducible MIC data for all strains, including the CLSI quality-control strain, were obtained. The MIC data for each strain are summarized in Table 1.

<table>
<thead>
<tr>
<th>C. difficile strain</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fidaxomicin</td>
</tr>
<tr>
<td>ATCC 43255</td>
<td>0.125</td>
</tr>
<tr>
<td>UK-14</td>
<td>0.5</td>
</tr>
<tr>
<td>CD196</td>
<td>0.125</td>
</tr>
<tr>
<td>UK-1</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Effects of drugs on toxin production

Both fidaxomicin and OP-1118 demonstrate bactericidal activity versus C. difficile strains; sub-MIC (1/4× or 1/8× MIC) drug levels were therefore used during toxin production studies to ensure that viable cell density was not affected. As shown in Figure 2(a) and Figure 3(a), addition of drugs at the early stationary phase of growth (24 h for ATCC 43255 strain and 10 h for the UK-14 strain) did not affect the growth of viable bacteria, but demonstrated an inhibitory effect on toxin production.

TcdA and TcdB levels for the ATCC 43255 strain, expressed as the percentage present versus the no-drug control, are depicted in Figure 2(b) and Figure 2(c), respectively. Both fidaxomicin and OP-1118 (at 1/4× MIC) drastically inhibited production of toxins A and B (by >80%). Suppression of both toxins continued even following 1 week of culture and was concentration-dependent for fidaxomicin. In contrast, vancomycin did not inhibit expression of either toxin in this strain.

Similar findings were observed with the UK-14 strain, which produced much lower levels of toxins, especially for the...
fidaxomicin- and OP-1118-treated samples, which were mostly below the LLOQ (thus these values represent, in general, an upper boundary for the concentrations present). However, by setting undetectable concentrations at the LLOQ, the percentage of toxin in each treatment could be calculated relatively between different treatment groups. As shown in Figure 3(b and c), both fidaxomicin and OP-1118 were able to reduce TcdA and TcdB levels by ≥60%. In contrast, vancomycin and metronidazole did not inhibit TcdA or TcdB levels in this strain. Increasing the vancomycin concentration (1× MIC), which did not affect cell viability during the early stationary phase as determined by cfu, still had no inhibitory effect on levels of toxin (data not shown).

Figure 2. Growth (a) and toxin levels (b and c) for C. difficile strain ATCC 43255. The data were obtained from at least two independent runs (at least three independent runs for fidaxomicin and vancomycin at 1/4× MIC). FDX, fidaxomicin; VAN, vancomycin.
Effect of drugs on toxin gene expression

To assess the effect of drug treatment on toxin locus gene expression, we measured the accumulation of tcdR (positive regulator of toxin genes), tcdB and tcdA mRNAs in cells treated with drugs at the onset of stationary phase (T₀) in two strains (UK-1 and CD 196). As shown in Table 2, accumulation of tcdB and tcdA mRNAs, coding for the two major toxins, was strongly inhibited by the addition of fidaxomicin (0.25 mg/L) or OP-1118.
By contrast, addition of vancomycin at T0 had little or no inhibitory effect on subsequent accumulation of \(tcdB\) and \(tcdA\) mRNAs. The accumulation of \(tcdR\) mRNA in strain UK-1 was similarly inhibited by fidaxomicin and OP-1118, but much less so by vancomycin.

Table 2. Effects of drugs on transcript levels of pathogenicity locus (PaLoc) genes

<table>
<thead>
<tr>
<th>Time</th>
<th>No drugs</th>
<th>Fidaxomicin</th>
<th>OP-1118</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C.) difficile UK-1 expression ratio (tcdB/rpoC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_0)</td>
<td>1 (0.36–2.75)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_2)</td>
<td>44.63 (16.25–122.47)</td>
<td>0.11 (0.02–0.56)</td>
<td>0.65 (0.34–1.23)</td>
<td>63.31</td>
</tr>
<tr>
<td>(T_4)</td>
<td>130.89 (54.90–312.06)</td>
<td>0.35 (0.09–1.24)</td>
<td>1.57 (0.79–3.13)</td>
<td>152.68</td>
</tr>
<tr>
<td>(C.) difficile UK-1 expression ratio (tcdA/rpoC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_0)</td>
<td>1 (0.36–2.77)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_2)</td>
<td>24.27 (0.19–64.05)</td>
<td>0.06 (0.01–0.43)</td>
<td>0.14 (0.04–0.43)</td>
<td>37.53</td>
</tr>
<tr>
<td>(T_4)</td>
<td>54.50 (23.78–124.91)</td>
<td>0.07 (0.01–0.39)</td>
<td>0.18 (0.09–0.37)</td>
<td>71.34</td>
</tr>
<tr>
<td>(C.) difficile UK-1 expression ratio (tcdC/rpoC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(T_0)</td>
<td>1 (0.37–2.64)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_2)</td>
<td>2.14 (0.98–4.69)</td>
<td>0.48 (0.13–1.70)</td>
<td>0.27 (0.07–1.09)</td>
<td>2.91</td>
</tr>
<tr>
<td>(T_4)</td>
<td>3.16 (1.51–6.63)</td>
<td>1.72 (0.70–4.23)</td>
<td>1.14 (0.27–4.81)</td>
<td>3.21</td>
</tr>
<tr>
<td>(C.) difficile CD196 expression ratio (tcdA/16S) rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_0)</td>
<td>1 (0.33–3.02)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_2)</td>
<td>7.80 (2.84–21.41)</td>
<td>0.19 (0.02–1.92)</td>
<td>0.13 (0.02–0.74)</td>
<td>2.91</td>
</tr>
<tr>
<td>(T_4)</td>
<td>26.86 (8.99–80.22)</td>
<td>0.05 (0.01–0.37)</td>
<td>0.03 (0.02–0.07)</td>
<td>1.55 (0.69–3.44)</td>
</tr>
<tr>
<td>(C.) difficile CD196 expression ratio (tcdB/16S) rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_0)</td>
<td>1 (0.50–1.99)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_2)</td>
<td>12.15 (5.02–29.40)</td>
<td>0.26 (0.09–0.77)</td>
<td>1.64 (0.61–4.43)</td>
<td>2.23 (1.07–4.64)</td>
</tr>
<tr>
<td>(T_4)</td>
<td>42.39 (17.87–100.55)</td>
<td>0.16 (0.07–0.34)</td>
<td>0.98 (0.76–1.26)</td>
<td>5.21 (2.17–12.66)</td>
</tr>
</tbody>
</table>

Strains UK-1 and CD196 were grown in TY medium and drugs were added as indicated at the end of the exponential growth phase (\(T_0\)). Samples were removed before drug addition (\(T_0\)) or 2 or 4 h later. Levels of PaLoc transcripts were determined by quantitative RT–PCR and normalized to levels of \(rpoC\) mRNA. Fidaxomicin was added at 0.25 mg/L, OP-1118 at 2.5 mg/L and vancomycin at 2.5 mg/L. qPCR was performed in triplicate for each sample. Mean and standard deviation values reflect results from at least three biological replicates, except for vancomycin treatment.

Table 3. Effect of drugs on transcript levels of toxin genes

<table>
<thead>
<tr>
<th>Time</th>
<th>No drugs</th>
<th>Fidaxomicin</th>
<th>OP-1118</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C.) difficile UK-1 expression ratio (tcdA/16S) rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_2)</td>
<td>1 (0.39–2.51)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_4)</td>
<td>1.46 (0.17–12.44)</td>
<td>0.01 (0.00–0.02)</td>
<td>0.04 (0.02–0.07)</td>
<td>1.55 (0.69–3.44)</td>
</tr>
<tr>
<td>(C.) difficile UK-1 expression ratio (tcdB/16S) rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_2)</td>
<td>1 (0.06–14.58)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(T_4)</td>
<td>2.48 (0.96–6.44)</td>
<td>0.03 (0.01–0.07)</td>
<td>0.09 (0.04–0.17)</td>
<td>2.23 (1.07–4.64)</td>
</tr>
</tbody>
</table>

Strain UK-1 was grown in TY medium and drugs were added as indicated at \(T_2\) (2 h after the end of the exponential growth phase). Samples were removed before drug addition (\(T_2\)) or 2 h later (\(T_4\)). Levels of \(tcdA\) and \(tcdB\) transcripts were determined by quantitative RT–PCR and normalized to levels of 16S rRNA. Fidaxomicin was added at 0.25 mg/L, OP-1118 at 2.5 mg/L and vancomycin at 2.5 mg/L. qPCR was performed in triplicate for each sample. Mean and standard deviation values reflect results from at least three biological replicates.

When fidaxomicin or OP-1118 was added at \(T_2\), the amounts of \(tcdA\) and \(tcdB\) mRNA decreased substantially in strain UK-1 by \(T_4\) (Table 3), indicating that addition of the drug after initiation of toxin gene expression prevented further accumulation of mRNAs.

(2.5 mg/L) at \(T_0\) in both strains. By contrast, addition of vancomycin at \(T_0\) had little or no inhibitory effect on subsequent accumulation of \(tcdB\) and \(tcdA\) mRNAs. The accumulation of \(tcdR\) mRNA in strain UK-1 was similarly inhibited by fidaxomicin and OP-1118, but much less so by vancomycin.
Discussion  
Fidaxomicin is a novel transcription inhibitor that demonstrates favourable attributes for the treatment of CDI. In a previous study, we reported that fidaxomicin and its major metabolite suppress sporulation in Clostridium difficile.\textsuperscript{17} The present study reveals additional attributes of fidaxomicin by demonstrating its inhibitory effect on toxin gene expression and toxin production by four strains of Clostridium difficile, including two hypervirulent NAP1 isolates (UK-14 and UK-1) as well as the ATCC 43255 strain; the latter is a potent toxin-producing strain, which was reported to be highly virulent in a mouse CDI model and exhibited higher pathological damage than other hypervirulent NAP1 strains in that study.\textsuperscript{23}

The basis for the presumed hypervirulence of UK-14 is not known. Under the laboratory conditions used in this study, UK-14, which is an epidemic NAP1 strain, did not produce as high levels of TcdA or TcdB as did ATCC 43255. Although we did not test the degree of cytotoxicity of UK-14, we have observed that this strain is extremely efficient in sporulation.\textsuperscript{17} It is likely that under in vivo, nutrient-limited conditions multiple attributes may lead to more virulent epidemic characteristics of strains. The heterogeneity of Clostridium difficile strains may not be surprising given that their genomes are rich in mobile genetic elements and are very diverse.\textsuperscript{24,25}

Regardless of toxin levels, both fidaxomicin and OP-1118, when added to stationary-phase cells, significantly suppressed the expression of tcdA and tcdB and the regulatory gene tcdR and strongly inhibited the production of both toxins A and B in UK-14 and ATCC 43255 strains. Inhibition was maintained during at least 1 week of culture. In contrast, vancomycin and metronidazole had no inhibitory effect on production of either toxin. The lack of inhibitory effect by vancomycin was consistent with its lack of effect on toxin gene expression in the UK-1 strain and with previous observations by other investigators, who have shown either no effect or increased toxin production, probably due to release of intracellular toxin, following treatment with vancomycin or metronidazole.\textsuperscript{10–12} The lack of inhibition by vancomycin when added to stationary-phase cells is not surprising given that vancomycin acts by blocking cell wall synthesis, a process restricted to growing cells.

It is unclear if inhibition of toxin production contributes to the observed lower recurrence rate with fidaxomicin treatment. Certainly, toxin-binding compounds and neutralizing anti-toxin antibody have been associated with lower recurrence rates of CDI\textsuperscript{26–28} Sub-MIC levels of fidaxomicin could provide additional protection by inhibiting further toxin production by any residual bacteria that may have remained following treatment. Indeed, in vivo findings, albeit with low numbers of subjects, indicated less frequent toxin positivity, 11–18 days post-infection, in fidaxomicin-treated subjects compared with that in vancomycin-treated patients.\textsuperscript{15}

In summary, our findings demonstrate that addition of fidaxomicin or its major metabolite, OP-1118, (but not vancomycin or metronidazole) to stationary-phase cells strongly suppresses the expression of the tcdA and tcdB genes, leading to inhibition of toxin production. Suppression of toxin production may contribute to the improvement in sustained clinical response observed for fidaxomicin.

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Transparency declarations  
F. B. and P. S. are employees of Optimer Pharmaceuticals, Inc. and own Optimer stock options. C. S. and A. G. were employed by Optimer Pharmaceuticals, Inc. L. B. and A. L. S. each received a grant from Optimer Pharmaceuticals, Inc. and are employees of Tufts University School of Medicine.

References  


