A new macrocyclic antibiotic, fidaxomicin (OPT-80), causes less alteration to the bowel microbiota of *Clostridium difficile*-infected patients than does vancomycin

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Clostridium difficile infection (CDI) is the most common identifiable cause of diarrhoea in hospitalized patients. Current therapies rely on the administration of metronidazole or vancomycin, which reduce vegetative populations of *C. difficile* in the bowel. Recurrence of the disease when treatment with these antibiotics ceases indicates that metronidazole and vancomycin affect not only *C. difficile* but also commensal populations that normally mediate competitive exclusion. Fidaxomicin is a new antibiotic that inhibits *C. difficile*. Our study shows that fidaxomicin had little effect on the composition of the faecal microbiota in terms of its major phylogenetic clusters. Notably, clostridial clusters XIVa and IV, and *Bifidobacterium*, were much less affected by fidaxomicin compared to vancomycin treatment. These findings help to explain the substantially reduced rates of relapse following treatment of CDI with fidaxomicin in recent clinical trials.

INTRODUCTION

Clostridium difficile infection (CDI) is the most common identifiable cause of diarrhoea in hospitalized patients (Calfee, 2008; Shannon-Lowe *et al.*, 2010). The incidence and severity of infections are increasing, so there is much interest in drug discovery and the development of new therapies in this field. Metronidazole and vancomycin are the current therapeutic options for the treatment of CDI, but these are considered to be inadequate because of decreased response rates to metronidazole and the recurrence of disease in patients (Miller, 2010). Up to 30% of patients who initially respond to metronidazole or vancomycin treatment experience a clinical recurrence following the cessation of antibiotic administration (Miller, 2010). Treatment with these antibiotics reduces vegetative *C. difficile* populations in the bowel, resulting in a clinical cure. Relapses occur due to the subsequent germination of *C. difficile* spores in the bowel and the proliferation of vegetative cells in the biome that, due to the concomitant reduction of microbiota populations by the antibiotics, lacks the regulatory phenomenon of competitive exclusion normally mediated by commensal bacteria (Hardin, 1960). Fidaxomicin (formerly known as OPT-80) is a newly

developed 18-membered-ring macrocyclic antibiotic that has good anti-*C. difficile* activity (MIC₉₀ 0.12 µg ml⁻¹) but is not inhibitory to commonly cultured bowel commensals (MIC₉₀ >1024 µg ml⁻¹; Louie *et al.*, 2009a). Fidaxomicin has been demonstrated to provide a clinical cure in phase 3 human trials with a significantly lower rate of disease recurrence than is seen with vancomycin (Miller, 2010). Culture-dependent studies have demonstrated that the *Bacteroides fragilis* group in faeces was not affected by fidaxomicin in therapeutic dosages and that the antibiotic was as effective as vancomycin in reducing *C. difficile* numbers (Louie *et al.*, 2009a). Traditional, selective culture-dependent studies are unable to detect a certain proportion of the faecal microbiota; therefore we have used culture-independent methods to investigate the impact of fidaxomicin on the composition of the faecal microbiota of patients relative to that of vancomycin.

METHODS

Faecal samples. Faecal samples were analysed from 23 patients with a mild to moderate CDI, recruited for a phase 2 dose-ranging clinical trial (Louie *et al.*, 2009b). The patients were adults with three or more loose, unformed bowel motions per day during the enrolment period, who were untreated, were without fever and had little abdominal pain. Patients were divided into three groups: eight patients administered 50 mg fidaxomicin twice daily for 10 days, seven patients administered 100 mg fidaxomicin twice daily for 10 days and eight patients administered 200 mg fidaxomicin twice daily for

Abbreviations: CDI, *Clostridium difficile* infection; Cy-5, indodicarbocyanine; FISH/FC, fluorescent *in situ* hybridization and flow cytometry; MDS, multi-dimensional scaling; TTGE, temporal temperature gradient electrophoresis.

Fig. 1. (a) Images produced by MDS showing distribution of TTGE profiles generated from faecal samples collected from fidaxomicin-treated patients at days 0 (green spheres), 7 (blue spheres), 10 (purple spheres) and 21 (red spheres). Note the absence of clear separation of profiles collected on different days. (b) All samples plotted. Profiles from healthy subjects and day 365 samples from ex-fidaxomicin-treated patients (all represented by red spheres) tend to cluster together. Profiles from antibiotic-treated patients are loosely distributed although profiles from vancomycin-treated patients (days 0, 10, 21; blue spheres) tend to segregate from those of fidaxomicin-treated patients (days 0, 7, 21; green spheres).

10 days. Twenty-three samples were collected on day 0 (prior to treatment of CDI), 22 on days 7, 10 and 21, and 9 on day 365 (one year post-treatment samples that were included as internal 'normal' controls). Additionally, faecal samples obtained from eight patients that were administered 125 mg vancomycin four times daily for 10 days were included in the analysis. These patients were matched demographically and symptomatically with the fidaxomicin patients (see Louie *et al.*, 2009b for demographics) and faecal collections were made at days 0, 10 and 21. None of the *C. difficile*-infected patients received concomitant antibiotics. Faecal samples were also obtained from eight healthy subjects (single samples). The stool samples were frozen within approximately 1 h of collection and stored at -80°C prior to bacteriological analysis. Approval of the clinical work was obtained from the Conjoint Health Ethics Board, University of Calgary. Participants gave their informed consent.

Generation and comparison of faecal microbiota profiles using temporal temperature gradient electrophoresis (TTGE).

Bacterial DNA was extracted from stool samples as described previously (Tannock *et al.*, 2004). Universal bacterial primers HDA1-GC and HDA2 were used to amplify the V3 region of the 16S rRNA gene by PCR, as described previously (Tannock *et al.*, 2004). Microbiota profiles were generated using the DCode universal mutation detection system apparatus (Bio-Rad) as described previously (Gore *et al.*, 2008). A reference sample with fragments distributed throughout the whole gel was included in gel runs to permit normalization and comparison of profiles. Gelcompar II (Allied Maths) was used to compare TTGE profiles by multi-dimensional scaling (MDS). MDS uses the similarity matrix as input which can be applied directly to pairwise-compared profile patterns.

Quantification of phylogenetic groups of bacteria in faeces by using fluorescent *in situ* hybridization and flow cytometry (FISH/FC). The proportions of seven phylogenetic groups commonly detected in human faeces were determined using oligonucleotides

Erec482, Clep866, Bac303, Bif164, Ato291, Enter1432 and Lab158. These probes detect bacterial members of clostridial cluster XIVa, clostridial cluster IV, *Bacteroides-Prevotella*, *Bifidobacterium*, *Atopobium*, enterobacteria and *Enterococcaceae-Lactobacillaceae* respectively. Details of these oligonucleotide probes were given by Lay *et al.* (2005). The proportions of each phylogenetic group were measured in comparison to the total bacterial community, which was determined using propidium iodide and probe Eub 338 covalently

Fig. 2. Comparison of clostridial cluster XIVa populations determined by FISH/FC in the faeces of patients treated with 50, 100 or 200 mg fidaxomicin twice daily for 10 days. Medians and inter-quartile ranges are shown; seven or eight patients were used per group. The populations did not differ between groups (Mann-Whitney test $P > 0.05$).

Fig. 3. Quantification of bacterial populations in faeces by FISH/FC. Results are the means of multiple individuals in each sampling group. Error bars in (a) and (b) represent standard error. (a, c) Results from fidaxomicin-treated patients (pooled data from all doses). The numbers of faecal samples per sampling time were 23 at day 0, 22 at days 7, 10 and 21, and 9 at day 365. (b, d) Results obtained from the eight vancomycin-treated patients. (c, d) Graphs were plotted to produce area fill curves (GraphPad Prism, Graphpad software) that emphasize outgrowth of enterobacteria (light shading) coincident with decrease in proportion of clostridial cluster XIVa (dark shading) during treatment with vancomycin. (e) Data from eight healthy subjects. Oligonucleotide probes used to determine the groups are labelled 1–6: 1, Erec482 detects members of clostridial cluster XIVa; 2, Clep866, clostridial cluster IV; 3, Bac303, *Bacteroides*–*Prevotella*; 4, Bif164, *Bifidobacterium*; 5, Ato291, *Atopobium*; 6, Enter1432, enterobacteria.

linked at the 5' end to FITC. The probes specific to particular bacterial groups were linked at the 5' end to the sulfoindocyanine dye indodicarbocyanine (Cy-5). Positive and negative control probes (Eub 338 and non-Eub 338) were labelled with Cy-5. Competitor oligonucleotides were unlabelled. The probes were purchased from MWG Biotech. FISH/FC procedures were performed as described previously (Lay *et al.*, 2005). The Mann–Whitney test was used for statistical analysis.

Quantification of *C. difficile* in faeces using quantitative PCR.

Real-time quantitative PCR was carried out using an ABI 7500 Fast System in MicroAmp Fast Optical 96-well plates with optical adhesive film (Applied Biosystems). Primers targeting the *C. difficile* 16S rRNA gene were those previously described by Rinttilä *et al.* (2004) and were purchased from Invitrogen. All reactions were carried out in a final volume of 20 µl containing 1 × Fast SYBR Green PCR Mastermix (Applied Biosystems) and 300 nM of each primer. Template DNA

was extracted from stool samples as described above and further cleaned using Qiagen DNeasy blood and tissue spin columns (Qiagen); 5 µl of the DNA was then added to the Mastermix. The thermocycling profile consisted of an initial activation of the polymerase at 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Fluorescence levels were measured after the 60 °C annealing/extension step. A melt curve was generated to analyse product specificity. Standard curves were generated using genomic DNA isolated from *C. difficile* ATCC 9689^T by using the Gram-positive bacteria protocol of the Qiagen DNeasy blood and tissue kit. The standard DNA was quantified spectrophotometrically using a Nanodrop 1000 (Thermo Scientific) and diluted in 10-fold steps from 5×10^6 to 5×10^1 genomes per reaction, which were calculated on the basis of one copy of the target gene per genome. All reactions were carried out in duplicate and were run twice on separate plates. No-template controls and a further negative control containing 5×10^4 genomes of *Clostridium perfringens* ATCC 13124^T DNA were also included on each plate.

RESULTS

Comparison of TTGE profiles

Measurement of the similarity between profiles by MDS analysis did not reveal any clustering of the microbiota profiles of samples collected from fidaxomicin-treated patients at days 0, 7, 10 or 21 (Fig. 1a). MDS analysis showed that the profiles of samples from fidaxomicin-treated patients, vancomycin-treated patients and untreated controls tended to cluster separately, although profiles from fidaxomicin-treated subjects were more loosely distributed (Fig. 1b). Overall, MDS analysis indicated that the composition of the faecal microbiota was differentially altered by antibiotic treatment relative to healthy and day 365 sample controls.

Alterations in microbiota compositions detected by FISH/FC

The compositions of the faecal microbiota of patients administered different doses of fidaxomicin were similar for all phylogenetic clusters ($P > 0.05$); data for clostridial cluster XIVa as a proportion of the total microbiota in these patients are shown as an example in Fig. 2. Data obtained from fidaxomicin-treated patient samples were

therefore pooled (all three doses) for simplicity because all doses of fidaxomicin affected microbiota composition in the same way. Pooled results are shown in Fig. 3(a) and Table 1. Clostridial cluster XIVa and clostridial cluster IV populations increased during and after the fidaxomicin treatment period. Clostridial cluster XIVa populations attained levels similar to those of the control faeces by day 10 (Fig. 3a, c, e; $P > 0.05$). In contrast, vancomycin treatment greatly reduced the proportions of the clostridial clusters, and also of bifidobacteria, by day 10 of the treatment period ($P < 0.05$). Outgrowth of enterobacteria coincided with the decrease in other phylogenetic groups (Fig. 3b, d). Samples from fidaxomicin-treated patients that had been administered the antibiotic one year previously had a bacterial composition resembling that of the control faeces (Fig. 3a, e). Bacteria detected by the Lab 158 probe include mainly lactobacilli and enterococci. The proportions of these bacteria did not vary during the fidaxomicin treatment period, but increased during vancomycin treatment ($P < 0.05$), coincident with the decrease in proportions of clostridial clusters and bifidobacteria (Table 1). Overall, vancomycin treatment was characterized by a decrease in the proportions of obligately anaerobic bacteria that normally populate the human colon, and an outgrowth of facultatively anaerobic and microaerobic bacterial groups during the treatment period.

C. difficile populations in faeces

C. difficile populations were detected in 19 out of the 23 fidaxomicin-treated patients and seven out of the eight vancomycin-treated patients at day 0 (Fig. 4). One patient treated with vancomycin had detectable *C. difficile* at day 10, whereas four or five patients treated with fidaxomicin harboured these bacteria at days 7, 10 and 21. Three of these patients had received the lowest dose (50 mg) of fidaxomicin. This observation vindicated the selection of the higher dosage of 200 mg fidaxomicin twice daily for clinical usage. Both vancomycin and fidaxomicin are not absorbed from the bowel and observation of 1100 patients administered 400 mg fidaxomicin daily has not revealed adverse effects (Louie *et al.*, 2009b; T. Louie, unpublished clinical observations).

Table 1. Quantification of *Enterococcaceae*–*Lactobacillaceae* (probe Lab158) as a proportion of the total faecal microbiota

Data are means $\pm$ SE of percentage values. ND, No samples; NA, not applicable.

Treatment	Percentage of total faecal microbiota on day:			
	0	7	10	21
Fidaxomicin	4.06 $\pm$ 1.37	5.93 $\pm$ 2.03	6.40 $\pm$ 2.36	7.96 $\pm$ 3.18
Vancomycin	6.04 $\pm$ 2.66	ND	27.27 $\pm$ 4.53	18.68 $\pm$ 6.23
None (healthy controls)	4.72 $\pm$ 1.50	NA	NA	NA

Fig. 4. *C. difficile* populations in faeces of fidaxomicin (OPT)- and vancomycin (VAN)-treated patients. Quantities of *C. difficile* in day 10 and day 21 faecal samples from patients treated with 50 mg fidaxomicin doses are indicated by arrows. *C. difficile* was not detected in day 21 samples obtained from vancomycin-treated patients.

DISCUSSION

The composition of the faecal microbiota is considered to reflect microbiological phenomena occurring in the distal human colon (Moore *et al.*, 1978). The colonic microbiota has a relatively stable composition due to the self-regulatory activities of the bacterial community and the ability of its members to obtain carbon and energy sources from a wide variety of substrates that may vary in availability from day to day (Gill *et al.*, 2006). *C. difficile* infection results in marked diarrhoea that may become life threatening. Broad-spectrum antibiotic treatment that predisposes patients to CDI, and the purging action of diarrhoea, diminish the numbers of bacteria in the colon and, as shown in our day 0 data, reduces the proportions of the clostridial groups that normally predominate in the human bowel. Vancomycin effectively inhibits the growth of *C. difficile*, resulting in the recovery of the patient, but it also has devastating effects on the composition of the microbiota. Such perturbations of bacterial communities are likely to provide vacant niches that can be filled by otherwise subdominant species. Thus, in some patients, the effects produced by vancomycin on the bowel microbiota allow the germination of *C. difficile* spores and proliferation of vegetative cells in the bowel, causing a re-emergence of *C. difficile* populations and, hence, a recurrence of diarrhoeal disease as recorded in clinical studies (Louie *et al.*, 2009b). Stool transplantation has been proposed as a means of quickly 'normalizing' the bowel microbiota but thorough investigations have not yet been completed (Rubin *et al.*, 2009). Fidaxomicin, as reported here, has a much less marked effect on the members of clostridial clusters XIVa and IV and bifidobacteria than does

vancomycin. Emphasis, to date, has been placed on the lack of antibacterial activity of fidaxomicin against Gram-negative bacteria (Louie *et al.*, 2009a) but our study has shown that Gram-positive commensals are also relatively unaffected. By sparing the normally predominant members of the microbiota, fidaxomicin treatment appears to minimize the disruption of competitive exclusion: in phase 3 trials of the antibiotic, fidaxomicin patients had a 47% lower recurrence rate of CDI versus vancomycin ($P=0.004$) (Miller, 2010). Moreover, the bowel microbiota is highly interactive with the host, which can have important physiological outcomes (Hooper, 2004; Bäckhed *et al.*, 2004). Therefore, antibiotics that produce a cure while having minimal effects on the composition of the microbiota are desirable. Their use should ensure that normal physiological processes are restored quickly with minimal consequences to the human-microbe balance that underpins the healthy bowel biome. Overall, our observations affirm the hypothesis that fidaxomicin is a narrow-spectrum and directed therapeutic agent for CDI. As shown in phase 3 trials, the antibiotic meets both end-point requirements of treatment: cure and an absence of recurrent disease in a large proportion of patients.

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REFERENCES

- Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F. & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* **101**, 15718–15723.
- Calfee, D. P. (2008). *Clostridium difficile*: a reemerging pathogen. *Geriatrics* **63**, 10–21.
- Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M. & Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* **312**, 1355–1359.
- Gore, C., Munro, K., Lay, C., Bibiloni, R., Morris, J., Woodcock, A., Custovic, A. & Tannock, G. W. (2008). *Bifidobacterium pseudocatenulatum* is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants. *J Allergy Clin Immunol* **121**, 135–140.
- Hardin, G. (1960). The competitive exclusion principle. *Science* **131**, 1292–1297.
- Hooper, L. V. (2004). Bacterial contributions to mammalian gut development. *Trends Microbiol* **12**, 129–134.
- Lay, C., Rigottier-Gois, L., Holmstrom, K., Rajilic, M., Vaughan, E. E., de Vos, W. M., Collins, M. D., Thiele, R., Namsolleck, P. & other authors (2005). Colonic microbiota signatures across five northern European countries. *Appl Environ Microbiol* **71**, 4153–4155.
- Louie, T. J., Emery, J., Krulicki, W., Byrne, B. & Mah, M. (2009a). OPT-80 eliminates *Clostridium difficile* and is sparing of *Bacteroides* species during treatment of *C. difficile* infection. *Antimicrob Agents Chemother* **53**, 261–263.

Louie, T., Miller, M., Donskey, C., Mullane, K. & Goldstein, E. J. C. (2009b). Clinical outcomes, safety and pharmacokinetics of fidaxomicin in a phase 2 trial of patients with *Clostridium difficile* infection. *Antimicrob Agents Chemother* **53**, 223–228.

Miller, M. (2010). Fidaxomicin (OPT-80) for the treatment of *Clostridium difficile* infection. *Expert Opin Pharmacother* **11**, 1569–1578.

Moore, W. E. C., Cato, E. P. & Holdeman, L. V. (1978). Some current concepts in intestinal bacteriology. *Am J Clin Nutr* **31** (Suppl. 10), S33–S42.

Rinttilä, T., Kassinen, A., Malinen, E., Krogus, L. & Palva, A. (2004). Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl Microbiol* **97**, 1166–1177.

Rubin, T. A., Gessert, C. E. & Aas, J. (2009). Stool transplantation for older patients with *Clostridium difficile* infection. *J Am Geriatr Soc* **57**, 2386–2387.

Shannon-Lowe, J., Matheson, N. J., Cooke, F. J. & Aliyu, S. H. (2010). Prevention and medical management of *Clostridium difficile* infection. *BMJ* **340**, c1296.

Tannock, G. W., Munro, K., Bibiloni, R., Simon, M. A., Hargreaves, P., Gopal, P., Harmsen, H. & Welling, G. (2004). Impact of consumption of oligosaccharide-containing biscuits on the fecal microbiota of humans. *Appl Environ Microbiol* **70**, 2129–2136.

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