Effects of piperacillin/tazobactam on *Clostridium difficile* growth and toxin production in a human gut model

Simon D. Baines, Jane Freeman and Mark H. Wilcox*

Department of Microbiology, University of Leeds and The General Infirmary, Old Medical School, Leeds LS1 3EX, UK

Received 3 September 2004; returned 19 November 2004; revised 9 March 2005; accepted 14 March 2005

**Objectives:** *Clostridium difficile* infection (CDI) is a major cause of morbidity in the nosocomial environment. Antimicrobial agents such as the third-generation cephalosporins, lincosamides and aminopenicillins are well known for their propensity to induce CDI, but the definitive reasons why remain to be elucidated. Despite their broad spectrum of activity against both aerobic and anaerobic bacteria, the ureidopenicillins remain a class of antimicrobials infrequently associated with the development of CDI.

**Methods:** We used a triple-stage chemostat model that simulates the human gut to study the effects of the ureidopenicillin/β-lactamase inhibitor combination piperacillin/tazobactam on gut bacterial populations and *C. difficile*.

**Results:** Piperacillin/tazobactam rapidly reduced all enumerated gut bacterial populations (including bacteroides, bifidobacteria and lactobacilli) below the limits of detection by the end of the piperacillin/tazobactam instillation period. Despite such widespread disruption of gut bacterial populations, *C. difficile* populations remained principally as spores, with no sustained proliferation or high-level cytotoxin production observed.

**Conclusions:** Factors other than reduced colonization resistance must be responsible for determining whether CDI develops following antimicrobial administration. We believe the gut model is a promising approach for the study of *C. difficile* pathogenesis reflecting *in vivo* events likely to occur in CDI.

Keywords: colonization resistance, pathogenesis, germination, spores

Introduction

*Clostridium difficile* is a major cause of morbidity particularly among elderly, hospitalized patients. It is the aetiologic agent of pseudomembranous colitis (PMC), and its major toxin(s) can be found in 95% of cases of PMC, and 30% of antibiotic-associated colitis (AAC).1,2 *C. difficile* infection (CDI) occurs almost exclusively as a result of antimicrobial administration and is thought to occur at least in part due to impairment of gut colonization resistance following antibiotic exposure.3–6 Clindamycin, third-generation cephalosporins (3GCs) and aminopenicillins are associated with the highest risk of development of CDI.6,7 Numerous studies have confirmed the link between 3GC administration and CDI.3–11 In contrast, reports linking ureidopenicillins with CDI are scarce, despite their broad spectrum of activity. Several studies have demonstrated that ureidopenicillins, with or without a β-lactam inhibitor, are significantly less likely to predispose to CDI than cephalosporins.12–14 For example, we found in a prospective cross-over study a 7-fold excess risk of developing CDI in elderly patients following cefotaxime compared with piperacillin/tazobactam therapy.12 The increased propensity of particular antimicrobials to induce CDI remains poorly understood, despite being the subject of a number of investigations.15–18 A recent study of the effects of sub-lethal concentrations of vancomycin, metronidazole, amoxicillin, clindamycin, cefoxitin and ceftriaxone upon growth and toxin A production *in vitro* reported no consistent relationship between antibiotics and growth or toxin production by *C. difficile*.18 Investigations in a test tube model of CDI failed to demonstrate any reproducible differences in growth, toxin production or sporulation between cefotaxime-, piperacillin/tazobactam-, ciprofloxacin-, or non-antibiotic-exposed faecal emulsions.19 We recently reported the use of a triple-stage chemostat gut model that is primed with pooled faeces

*Corresponding author. Tel: +44-113-392-6818; Fax: +44-113-233-5649; E-mail: mark.wilcox@leedsth.nhs.uk
and simulates the human gut. We demonstrated marked effects of cefotaxime and its desacetyl metabolite on *C. difficile* germination and subsequent cytotoxin production, compared with antibiotic-free control periods. In addition, the deleterious effects of cefotaxime and desacetyl cefotaxime on bacteroides suggested a possible role for these bacteria in colonization resistance. In this study, we examined the response of *C. difficile* and gut microflora in the gut model following exposure to no antibiotic and therapeutic concentrations of piperacillin/tazobactam.

**Materials and methods**

All anaerobic culture and manipulations were performed in a MK 3 Anaerobic Workstation (Don Whitley Scientific, Shipley, UK) at 37°C.

**Triple-stage chemostat gut model**

Macfarlane et al. validated a triple-stage chemostat model of the human gut based on physiological and microbiological measurements from the intestinal contents of sudden death victims. The system was designed to reproduce the spatial, temporal, nutritional and physicochemical characteristics of the proximal to distal bowel. Briefly, the model consisted of three vessels (V1, V2 and V3) under automatic pH and temperature control, and continuously sparged with oxygen-free nitrogen. The vessels operated in a weir cascade system that ran into a waste unit. Vessel 1 was supplied with growth medium at a controlled rate by a peristaltic pump. The vessels were maintained at pH 5.5, 6.2 and 6.8, respectively to reflect the increasing alkalinity of the colon from proximal to distal. Thus vessel 1 maintained at pH 5.5, 6.2 and 6.8, respectively to reflect the increasing alkalinity of the colon from proximal to distal.

Vessels 2 and 3, which operated at 300 mL each, reflected the more neutral, low substrate environment of the proximal colon. Vessel 1 had an operating volume of 280 mL and was designed to reflect the low pH, high substrate environment of the proximal colon. Vessels 2 and 3, which operated at 300 mL each, reflected the more neutral, low substrate environment of the distal colon. Foaming was prevented by the addition of 0.5 mL of polypropylene glycol (VWR International, Lutterworth, UK). The model was allowed to equilibrate for ~14 days in respect of bacterial populations, at a system retention time of 66.7 h (V1 = 16.7 h, V2 = 25 h, V3 = 25 h).

**Collection of human faecal material and inoculation of the gut model**

Human faeces for the inoculation of the gut model were collected from elderly volunteers (aged >65 years) with no history of antibiotic therapy for 2 months prior to sampling. Duplicate faecal samples were transported in sealed anaerobic bags at 4°C (Becton Dickinson, Sparks, MD, USA) and placed in an anaerobic cabinet within 12 h of collection. Samples were screened for the presence of *C. difficile* by 48 h anaerobic culture onto CCEYL agar [Braziers CCEY agar (Bioconnections, Leeds, UK) supplemented with 5 mg/L lysozyme and 2% lysed horse blood]. *C. difficile*-negative samples were pooled to produce ~50 g of faeces. Pooled faeces were emulsified in 500 mL of pre-reduced PBS, stomached and coarse filtered through sterile muslin to give a smooth 10% faecal slurry.

Each vessel was filled to approximately two-thirds volume with the 10% (w/v) faecal slurry. Vessel 1 was then primed with growth medium to a final volume of 280 mL, following which the media pump was started to allow V2 and V3 to be fed in cascade from V1. At the appropriate sampling frequency (Table 1), gut bacterial populations (periods A–D) and *C. difficile* total counts, spore counts and cytotoxin (period B onwards) were evaluated in all vessels of the gut model.

**Gut model growth medium**

All constituents for the gut model growth medium were supplied by Sigma–Aldrich (Poole, UK). The medium constituents were (g/L): peptone–Aldrich (Poole, UK). The medium constituents were (g/L): peptone 2.0, yeast extract 2.0, NaCl 0.1, K2HPO4 0.04, KH2PO4 0.04, MgSO4.7H2O 0.01, CaCl2.2H2O 0.01, NaHCO3 2.0, haemin 0.005, cysteine HCl 0.5, bile salts 0.5, glucose 0.4, arabinogalactan 1.0, pectin 2.0, starch 3.0. Liquid additions were as follows: vitamin K1 10 μg/L, Tween 80 0.2%. After autoclaving, glucose (0.4 g/L), and resazurin anaerobic indicator (0.005 g/L) were added through a sterile filtration device. The medium was pre-reduced by sparging with oxygen-free nitrogen overnight before use.

**Enumeration of *C. difficile* spores**

Two millilitre samples were removed from each vessel and 500 μL was treated with an equal volume of 96% ethanol (VWR International, Lutterworth, UK) for 1 h. Alcohol-shocked suspensions were then serially 10-fold diluted to 10−8 in pre-reduced peptone water (Sigma–Aldrich, Poole, UK). Triplicate CCEYL plates were inoculated with 20 μL of each dilution. Plates were incubated anaerobically for 48 h, and single colonies were counted.

**Enumeration of faecal bacteria**

Five hundred microlitres of each 2 mL sample was serially diluted in 4.5 mL of pre-reduced peptone water to 10−9, in an anaerobic cabinet. The following selective agars were inoculated in triplicate

**Table 1. Time periods during experiment**

<table>
<thead>
<tr>
<th>Time period</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (PT experiment)</td>
<td>1–19</td>
<td>steady state</td>
<td>20–26 C. difficile spores added</td>
<td>27–33 C. difficile spores added+PT tds</td>
</tr>
<tr>
<td>Day (control antibiotic-free experiment)</td>
<td>1–13</td>
<td>steady state</td>
<td>14–20 C. difficile spores added</td>
<td>21–27 C. difficile spores added</td>
</tr>
<tr>
<td>Sampling frequency</td>
<td>every 2 days</td>
<td>daily</td>
<td>daily</td>
<td>daily</td>
</tr>
</tbody>
</table>

PT, piperacillin/tazobactam; tds, three times daily.

*Steady-state period was extended from 14 to 20 days.

---

C. difficile infection human gut model
C. difficile cytotoxin quantification

Five hundred microlitres of each 2 mL sample was centrifuged at 16 000 g and the supernatant removed. Supernatants were serially diluted to 10⁻⁷ in PBS, and 20 μL of each dilution added to VERO cell monolayers in a 96-well microtitre tray. Trays were incubated at 37°C in 5% CO₂, and examined after 24 and 48 h under an inverted microscope. A positive reaction was indicated by cell rounding and parallel neutralization of effect with 20 μL of 10% Clostridium sordellii antitoxin (Prolab Diagnostics, Neston, UK). C. difficile cytotoxin titres were expressed in relative units (RU).

Preparation of C. difficile spores

Ten fresh blood agar plates were inoculated with C. difficile strain P24 (UK epidemic strain, PCR ribotype 1) and incubated anaerobically at 37°C for 10 days. All growth was removed and resuspended in 1 mL of sterile saline. An equal volume of 96% ethanol was added and the suspension incubated at room temperature for 1 h. Suspensions were centrifuged at 3000 g for 10 min, and the pellet resuspended in 1 mL of sterile saline. Spore suspensions were enumerated on CCEY agar, adjusted to ~ 10⁷ cfu/mL and stored at 4°C until required.

Preparation of piperacillin–tazobactam

Piperacillin/tazobactam was supplied by Wyeth-Ayerst (Maidenhead, UK). Piperacillin/tazobactam (Tazocin®) was prepared in 1 mL volumes, to achieve a final concentration of 358 mg/L when instilled into V1. This is approximately equivalent to mean bile piperacillin concentration reported by Westphal et al. after dosing individuals with a single intravenous (iv) infusion of piperacillin/tazobactam (4 g:0.5 g, piperacillin/tazobactam, respectively). Instillation took place every 8 h for 7 days in accordance with normal clinical dosing regimens.

Inoculation and antibiotic instillation to the gut model

The time periods for the experiments are shown in Table 1.

Piperacillin/tazobactam. The piperacillin/tazobactam gut model experiment was performed twice. After inoculation with faecal slurry, the model was allowed to equilibrate for ~14 days until bacterial populations were stable (period A). During period A, vessels were sampled every 2 days and every day thereafter. C. difficile spores were then added to V1 (~10⁷ cfu) and vessels were sampled daily for all bacterial species and cytotoxin (period B). A further 10⁷ cfu C. difficile spores were then added to the model. At this point, three times daily instillation of 358 mg/L piperacillin–tazobactam commenced and continued for 7 days (period C). Daily sampling was then performed for 7 days. Following cessation of antibiotic instillation, sampling continued for 7 days (period D).

Control. After inoculation with faecal slurry, the model was allowed to equilibrate for ~14 days until bacterial populations were stable (period A). During period A, vessels were sampled every 2 days and every day thereafter. C. difficile spores were then added to V1 (~10⁷ cfu) and vessels were sampled daily for all bacterial species and cytotoxin for 7 days (period B). A further 10⁷ cfu C. difficile spores were then added to the model. No further instillations were made, and sampling of vessels continued daily for 7 days.

Results

Effect of C. difficile spore introduction (period B)

Following instillation of C. difficile spores into the model, C. difficile total counts and spore counts remained equivalent in number, decreasing during this period of both piperacillin/tazobactam and non-antibiotic exposed control experiments (Figure 1a and b). This was observed in all three vessels. Cytotoxin titres reached only 1 RU (undiluted sample) in the piperacillin/tazobactam experiment, with no demonstrable cytotoxin in the non-antibiotic exposed control experiment (Figure 1a–c). Numbers of gut bacterial populations remained constant with no substantial fluctuations observed in either piperacillin/tazobactam or non-antibiotic exposed control experiments, in all vessels (Figure 2a and b).

Effect of piperacillin/tazobactam instillation versus no antibiotic instillation on C. difficile growth and toxin production (period C)

Apart from following piperacillin/tazobactam instillation, C. difficile total counts, spore counts, and cytotoxin titres in all vessels remained at levels similar to those observed in period B. Increased total C. difficile counts were observed after 5 days of piperacillin/tazobactam instillation, but only for a brief period (24 h), following which total counts decreased to levels equivalent to those of C. difficile spores (Figure 1a). During the same period of the non-antibiotic exposed control experiment, total C. difficile counts and spore counts remained equivalent and decreased concurrently, with no detectable cytotoxin (Figure 1b). This was observed in all three vessels.
C. difficile infection human gut model

Figure 1. (a) Effects of piperacillin/tazobactam (PT) on C. difficile total counts, spore counts and cytotoxin titres in vessel 3 of the gut model. (b) C. difficile total counts, spore counts and cytotoxin titres in vessel 3 of the gut model during antibiotic-free control experiment. (c) Comparison of the effects of PT, cefotaxime/desacetyl cefotaxime (CTX/dCTX) and no antibiotic instillation upon C. difficile cytotoxin titres in the gut model (*CTX results adapted from Freeman et al.26).
Figure 2. (a) Effect of piperacillin/tazobactam instillation upon gut bacterial populations in vessel 3 of the gut model. (b) Gut bacterial populations in vessel 3 of the gut model during antibiotic-free control experiment. (c) Effect of cefotaxime (CTX) instillation upon gut bacterial populations in vessel 3 of the gut model (CTX results adapted from Freeman et al. 20).
Effect of piperacillin/tazobactam instillation versus no antibiotic instillation upon gut bacterial populations

Following the instillation of piperacillin/tazobactam, viable counts of all faecal bacterial groups decreased to below the limit of detection in all vessels. Numbers of bacteroides, bifidobacteria, and Gram-positive cocci decreased to below the limit of detection after 2 days of piperacillin/tazobactam instillation (results not shown). Counts of lactobacilli, lactose fermenters, and total facultative anaerobes decreased below the limit of detection after 4 days of piperacillin/tazobactam instillation. Total anaerobe counts decreased from $10^9$ to $10^3$ cfu/mL by the end of period C (Figure 2a). In contrast, numbers of faecal bacteria remained steady in all vessels during the control experiment (Figure 2b).

Events following cessation of piperacillin/tazobactam instillation (period D)

Cessation of piperacillin/tazobactam instillation resulted in a marked decrease in total *C. difficile* counts and spore counts to below the limit of detection in vessel 1 (results not shown). Increases in total *C. difficile* with concurrent decreases in *C. difficile* spores were observed in vessel 2 (results not shown), while both an increase in total *C. difficile* counts and spore counts were observed in vessel 3 towards the end of period D (Figure 1a). *C. difficile* cytotoxin remained undetectable despite the rise in *C. difficile* numbers (Figure 1a and c). Cytotoxin was detected at a titre of 5 RU in the duplicate piperacillin/tazobactam experiment, on the final day of sampling (results not shown). In the non-antibiotic-exposed control, *C. difficile* total and spore counts remained approximately equivalent in numbers and decreased. No cytotoxin was detectable during this period (Figure 1b and c). This was observed in all vessels. After piperacillin/tazobactam instillation ended, populations of faecal bacteria recovered or slightly exceeded their pre-instillation numbers in vessels 2 and 3, excepting lactobacilli and bifidobacteria, which increased to slightly below pre-instillation levels (Figure 2a). Populations of faecal bacteria increased to pre-instillation levels in vessel 1, excepting lactobacilli (Figure 2a) and bifidobacteria, which remained unrecoverable.

Discussion

The propensity of certain antimicrobial agents to induce CDI is well documented, and yet remains poorly understood. Previous investigations have largely relied upon the hamster model of CDI, or *in vitro* test tube models. While these have provided valuable data, neither accurately reflect the human gut. Notably, data regarding potentially important bacterial genera must be interpreted with caution in light of the differences in microflora between humans and rodents. Human volunteer studies also have significant ethical considerations and do not permit collection of well-controlled data. Furthermore, previous *in vitro* studies have failed to reproduce results of *C. difficile* exposure to antibiotics that correlate with clinical observations. The inability to model CDI in a simple batch culture model indicates the multifactorial nature of CDI. We established a human gut model of CDI to address these issues, and to allow study of *C. difficile* in a gut reflective environment. The findings of this study clarify our understanding of *C. difficile* response to piperacillin/tazobactam exposure, a broad spectrum antimicrobial agent that is considered to have a low propensity to induce CDI.

We previously found that *C. difficile* remained in its spore state, following inoculation of the gut model, with no evidence of germination, exponential growth or toxin production in the absence of antibiotic administration. Similarly, in this study *C. difficile* numbers decreased steadily after inoculation, with no evidence of proliferation. This is also in agreement with previous studies in both faecal emulsions and the hamster model, which reported the inability of *C. difficile* to proliferate in the absence of antibiotic pressure. We occasionally detected 1 RU of cytotoxin prior to and during piperacillin/tazobactam instillation (periods B and C). Such low values are of doubtful significance and contrast sharply with cytotoxin levels associated with cefotaxime. As discussed previously, ideally, study of toxin gene expression is required alongside biological cytotoxin assays to understand fully low-level cytotoxin results. *C. difficile* spores were added to the gut model in relatively high numbers (10 spores) to avoid washout of the organism before colonization could be established. Despite this large inoculum, *C. difficile* remained in a quiescent spore state and indeed decreased in total numbers. We believe that this is analogous to the *in vivo* situation, where *C. difficile* rarely remains in the gut in the absence of antimicrobial pressure. It is currently not clear whether gut exposure to *C. difficile* usually precedes or follows antimicrobial administration, or whether these situations have differing risks of progression to CDI. During experiments with the gut model *C. difficile* spores were introduced 1 week before and during antimicrobial dosing. This acted as a control within individual experiments, showing that *C. difficile* could not germinate and produce toxin in the pooled faecal emulsion alone. This would not have been possible had we dosed with antibiotic before *C. difficile* exposure. However, investigation of this conundrum using the model may provide clinically valuable data.

Although increases in *C. difficile* numbers were observed during piperacillin/tazobactam dosing, these were not maintained despite substantial depletion of gut microflora (Figures 1a and 2a). Similar results for *C. difficile* were observed in the control antibiotic-free experiment (Figures 1b and 2b), but in the absence of gut flora depletion. Conversely, we previously observed *C. difficile* germination, proliferation and subsequent toxin production (Figure 1c) in response to cefotaxime administration, both alone and in combination with its major active metabolite, desacetyl cefotaxime. It has generally been accepted that *C. difficile* colonization and subsequent infection occur following the depletion of the normal gut microflora by the action of antimicrobials. However, our results demonstrate a notable lack of *C. difficile* germination, proliferation and subsequent toxin production despite profoundly deleterious effects of piperacillin/tazobactam on gut bacteria (Figures 1a and c and 2a). Indeed, the effects of cefotaxime/desacetyl cefotaxime were considerably less deleterious than those of piperacillin/tazobactam on gut microflora (Figure 2a and c). Nord et al. found decreased faecal viable counts of enterobacteria, enterococci, bifidobacteria, lactobacilli, clostridia, and Gram-positive cocci following piperacillin/tazobactam administration to hospitalized patients, but to a lesser degree than those reported in this study. Conversely, the lack of effect on *Bacteroides* spp. observed by Nord and colleagues contrasted markedly with the 8 log reduction seen in this study. This discrepancy may reflect fundamental difficulties with human volunteer studies.
which cannot be well controlled for gut flora composition or for actual bacterial exposure to antibiotic. Inter-patient piperacillin/tazobactam faecal concentrations can vary markedly. For example, both piperacillin and tazobactam could only be detected in four of 17 faecal samples in patients receiving piperacillin/tazobactam; the ratio of piperacillin to tazobactam in these four samples ranged from 2:1 to >11:1 despite 8:1 proportions at administration. It is possible that different ratios of piperacillin/tazobactam may have greater or lesser effects on gut flora. However, this theory was not investigated during this series of experiments. The profound effects of piperacillin/tazobactam on gut bacterial populations that we observed reflect the high piperacillin/tazobactam dosage (358 mg/L three times daily), which was chosen to mimic antimicrobial concentrations found in vivo. Such levels are substantially higher than the MIC of piperacillin/tazobactam for the C. difficile strain used in this study (2 mg/L). Thus, piperacillin/tazobactam may have directly prevented the germination of C. difficile spores in the gut model despite the markedly reduced numbers of competing bacterial species present at this time. This may explain the rise in cytotoxin titre (5 RU) observed in the duplicate piperacillin/tazobactam experiment, on the final day of sampling. The results of the control, piperacillin/tazobactam and cefotaxime/desacetyl cefotaxime gut model experiments suggest antimicrobial presence is necessary for C. difficile to produce toxin in detectable quantities. However, cytotoxin production did not take place immediately upon instillation of cefotaxime or cefotaxime/desacetyl cefotaxime into the gut model, implying that antimicrobial concentration affects toxin production. Germination and outgrowth may only be possible following a decrease in antimicrobial concentration below an inhibitory threshold. It is also possible that piperacillin/tazobactam may have a direct effect on C. difficile toxin production. Bioactive concentrations of piperacillin/tazobactam in the gut model were not assayed in this study due to storage difficulties. Alternatively, this late rise in toxin titre could have been due to experiment-specific physical phenomena, such as transient pH shifts. Although the model is pH controlled, there is considerable biofilm formation on the pH probes, that can adversely affect their function. Thus, we cannot exclude the possibility of transient pH shifts while manual cleaning of pH probes was carried out. We cannot be certain of the significance of the detection of a high cytotoxin titre on the final day of only one of two duplicate piperacillin/tazobactam gut model experiments. However, crucially, clinical experience suggests that CDI risk is reduced for piperacillin/tazobactam in comparison with cephaprin. These clinical data, and the relative timing of cytotoxin production in earlier gut model experiments with cefotaxime and desacetyl cefotaxime versus in only one experiment of the current piperacillin/tazobactam study, suggest that the latter observation is not directly related to piperacillin/tazobactam instillation.

The lack of germination, proliferation and, in particular, toxin production by C. difficile, despite the severely compromised gut flora is intriguing. Several studies have attempted to identify specific gut bacterial components that may be important in colonization resistance. Hopkins & Macfarlane demonstrated generally fewer Bacteroides spp. and Bifidobacterium spp. in elderly individuals, whereas CDI patients exhibited decreased bacteroides, Prevotella spp., and bifidobacterial populations. We also previously reported reduced numbers of bacteroides and bifidobacteria in the gut model following cefotaxime/desacetyl cefotaxime exposure, and suggested that these microorganisms may be particularly important in colonization resistance. Conversely, this study did not show any correlation between reduced bacteroides or bifidobacterial populations and concomitant growth and cytotoxin production by C. difficile. While the present data contradict the existence of a simple relationship between reduced numbers of bacteroides or bifidobacteria and increased levels of C. difficile and toxin, they do not preclude the possibility of a more complex interaction. If colonization resistance by host commensal gut bacteria was the exclusive factor in the prevention of CDI development, C. difficile may have been expected to proliferate and produce toxins in the absence of competing bacteria. Our results show that alternative factors must be influential in determining development of CDI. The balance between the effects of an antimicrobial on colonization resistance and on C. difficile itself may explain why some broad spectrum agents, such as piperacillin/tazobactam, are infrequently associated with CDI. If piperacillin/tazobactam is excreted in faeces at very low concentrations, the resultant effect on the indigenous gut flora will be less pronounced, and colonization by C. difficile may be prevented. At higher faecal piperacillin/tazobactam concentrations, the antibiotic will have a more profound effect on gut flora, but will also be active against C. difficile. Gerding recently suggested that antimicrobial resistance of C. difficile may be a factor in the development of CDI. Increased reports of fluoroquinolone-associated CDI may be explained by reduced susceptibility of C. difficile to these antibiotics.

The profoundly deleterious effect of piperacillin/tazobactam on gut flora was followed by a rapid recovery of all bacterial genera after cessation of dosing. Larson & Borriello demonstrated differences in the duration of susceptibility to CDI in the hamster model of infection following treatment with clindamycin, ampicillin, flucloxacillin and cefuroxime. No differences in duration of susceptibility were observed for ampicillin, flucloxacillin and cefuroxime. However, clindamycin-treated hamsters were susceptible to CDI for 73 days after a 3 mg dose, and the persisting loss of colonization resistance was postulated as a type of post-antibiotic effect. Our results indicate that piperacillin/tazobactam does not promote long-lasting depletion of gut bacterial populations. It is possible that bacterial biofilm on the vessel walls may have provided a protected reservoir for some gut bacterial populations during antibiotic dosing, allowing a rapid repopulation of the vessels following the cessation of antibiotic instillation. The inner surfaces of the gut model vessels accumulate profuse bacterial biofilms during the course of an experiment. Indeed, such biofilms are required for the maintenance of ecological balance within a continuous culture system. Biofilm matrix comprises extracellular polysaccharide, proteins, nucleic acids, polysaccharides and glycoproteins. Therefore, the potential exists for interaction between C. difficile toxins and biofilm constituents. Sequestration and subsequent release of toxin may account for the low-level cytotoxin titres observed both in the present and previous studies in the gut model. Cytotoxin titres observed during this study (1 RU) are very low in comparison with those seen after cefotaxime/desacetyl cefotaxime dosing (5 RU) (Figure 1e). High cytotoxin titres follow C. difficile germination and are probably analogous to CDI onset in vivo.
An intact normal gut flora is thought to present a natural barrier to colonization and subsequent infection by \textit{C. difficile}.\textsuperscript{15–17} Although the identification of the specific bacterial components responsible for colonization resistance to \textit{C. difficile} has received much attention over the past two decades,\textsuperscript{18,29} the importance of gut concentrations of antimicrobials and/or metabolites has largely been overlooked. This was partly due to the lack of a reliable and reproducible in \textit{vitro} model of \textit{C. difficile} infection. We believe the results presented here further demonstrate a successful in \textit{vitro} model of \textit{C. difficile} infection. While the gut model cannot mimic immunological or secretory events, it does provide a gut-reflective environment in which to study the interplay between \textit{C. difficile}, gut bacterial populations (i.e. colonization resistance to \textit{CDI}), and antimicrobial concentrations. Our data suggest that either the components responsible for colonization resistance during antimicrobial treatment are non-cultivatable, or that \textit{C. difficile} has a more complex pathogenesis than simply proliferation of \textit{C. difficile} following antimicrobial-induced gut microflora depletion. Finally, this predictive gut model offers the potential to evaluate new antimicrobials for their propensity to induce \textit{CDI}, and also to examine new therapeutics for this disease.

Acknowledgements

We thank Professor Glen Gibson and co-workers at the University of Reading for their help and advice in establishing the gut model in Leeds. We acknowledge the invaluable support and encouragement of Professor Roger Freeman and thank Shamham Qamar for technical assistance. We acknowledge the financial support of the BSAC and Wyeth-Ayerst.

References


