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Review

Ridinilazole: a novel therapy for *Clostridium difficile* infection

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ABSTRACT

Clostridium difficile infection (CDI) is the leading cause of infectious healthcare-associated diarrhoea. Recurrent CDI increases disease morbidity and mortality, posing a high burden to patients and a growing economic burden to the healthcare system. Thus, there exists a significant unmet and increasing medical need for new therapies for CDI. This review aims to provide a concise summary of CDI in general and a specific update on ridinilazole (formerly SMT19969), a novel antibacterial currently under development for the treatment of CDI. Owing to its highly targeted spectrum of activity and ability to spare the normal gut microbiota, ridinilazole provides significant advantages over metronidazole and vancomycin, the mainstay antibiotics for CDI. Ridinilazole is bactericidal against *C. difficile* and exhibits a prolonged post-antibiotic effect. Furthermore, treatment with ridinilazole results in decreased toxin production. A phase 1 trial demonstrated that oral ridinilazole is well tolerated and specifically targets clostridia whilst sparing other faecal bacteria. Phase 2 and 3 trials will hopefully further our understanding of the clinical utility of ridinilazole for the treatment of CDI.

1. Introduction

1.1. Overview and epidemiology

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacillus first identified in 1978 as the causative agent of pseudomembranous colitis in humans [1]. Symptoms of *C. difficile* infection (CDI) range from mild to moderate diarrhoea, often with cramping abdominal pain, to fulminant disease, which can manifest as severe diarrhoea, colitis, toxic megacolon, bowel perforation and sepsis [2].

Outbreaks of severe CDI in the USA and Canada in the early 2000s were attributed to the rapid emergence of a fluoroquinolone-resistant strain of *C. difficile* characterised as REA group BI, pulsed-field gel electrophoresis (PFGE) type NAP1, and PCR ribotype 027 (BI/NAP1/027) [3–5]. The rapid transcontinental spread of two distinct lineages of the BI/NAP1/027 epidemic strain has driven the increased frequency and severity of global CDIs [6]. CDI is now the leading cause of infective healthcare-associated diarrhoea [7]. A US surveillance study funded by the US Centers for Disease Control and Prevention (CDC) estimated the national incidence of CDI in 2011 at almost half a million cases with ca. 29,000 deaths within 30 days of initial diagnosis [8]. Epidemic *C. difficile* 027 remains the most common ribotype in the USA and accounts for approximately one-third of cases [9,10]. The overall prevalence of ribotype 027 in Europe has increased more than three-fold from 2008 to 2013, notably in Eastern European countries and in Germany [11]. Ribotype 027 and other hypervirulent strains, including ribotypes 078, 126 and 244, are associated with fulminant disease, increased risk of life-threatening complications and increased rates of mortality [12–15].

CDI has traditionally been thought of as a nosocomial infection, but the increasing frequency of community-associated CDI has underscored the growing threat of *C. difficile* transmission outside of the hospital setting [16–18]. A 2012 CDC report showed that 94% of CDI cases were associated with contact with the healthcare system, but in 75% of cases disease onset occurred in non-hospitalised patients and 52% of CDI cases were already present on admission [19]. Interestingly, and as yet unexplained, approximately one-third of community-associated CDIs do not have a history of recently prescribed antibiotics; other precipitating factors that could be relevant are foodstuffs, including antimicrobial substances therein, and other drugs that may alter the gut microbiome [20–23].

CDI disproportionately affects the elderly, with the incidence of CDI highest in those aged ≥65 years [8], and elderly individuals with

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CDI experience poorer treatment outcomes [24]. Antibiotic use remains the primary risk factor for CDI, and the majority of antibiotic classes in routine clinical use are associated to a greater or lesser degree with CDI [25,26].

1.2. Virulence factors

C. difficile secretes two major virulence factors, enterotoxin TcdA (toxin A) and cytotoxin TcdB (toxin B), which are the primary causes of inflammation and damage to the colonic mucosa, resulting in disease symptoms [27]. The cytotoxic effects of toxins A and B are mediated by their ability to glucosylate and inactivate epithelial cell GTPases such as Rac, Rho and Cdc42, which leads to alterations in cellular signalling that affect the actin cytoskeleton, disrupt barrier function and induce apoptosis. Some toxin B variants target Ras and Rap GTPases in place of Rac. Toxins A and B also contribute to tissue damage through their induction of pro-inflammatory cytokines such as interleukin 1-beta (IL-1β) [27]. Toxin B from ribotype 027 is associated with increased cytotoxicity, which may contribute to enhanced virulence [28]. Hypervirulent strains produce a third toxin known as *C. difficile* binary toxin (CDT). The role of this toxin in virulence has yet to be determined, although its presence may be linked to more severe disease [27,29,30]. Furthermore, there have been recent cases reported of CDI due to strains producing only CDT [31,32].

Hypervirulent strains are also potentially associated with increased spore production [33,34]. *C. difficile* spores play a major role in the pathogenesis of CDI [35]. Spores shed in the faeces of infected or colonised individuals are resistant to heat, acid and alcohol-based cleaners; thus, dormant spores can persist for months on environmental surfaces in healthcare settings and the community [36,37]. *C. difficile* spores act as the vector for infection, with transmission occurring through spore ingestion via the faeco–oral route [38].

1.3. Treatment options and disease recurrence

Treatment of CDI has largely been limited to the antibiotics metronidazole and vancomycin. Most current guidelines state that oral metronidazole is recommended for non-severe disease, whereas oral vancomycin is the preferred therapy for severe disease [39,40]. Notably, it has recently been shown that vancomycin results in superior clinical cure rates compared with metronidazole in patients both with non-severe and severe CDI [41,42]. This now raises questions about the place of metronidazole in the treatment options for CDI.

Fidaxomicin (Dificlir®, Astellas, Europe; Dificid®, Merck, USA) was approved in 2011 for the treatment of CDI. In phase 3 studies, fidaxomicin has been shown to be non-inferior to vancomycin on clinical response at the end of treatment (EOT) and superior to vancomycin on sustained clinical response to 28 days post-EOT. However, fidaxomicin was not shown to be superior to vancomycin on sustained clinical response in patients infected with BI/NAP1/027 strains [43,44].

Disease recurrence remains a central unmet medical need in the management of CDI. Following initial therapy with metronidazole or vancomycin, recurrence of CDI occurs in up to 30% of patients, and each episode of disease is associated with an increased risk of additional recurrent episodes. In a study of 163 patients with at least one recurrent CDI episode, the risk of subsequent episodes was 45% [45]. Following a third episode of CDI, recurrence rates may be >65% [46,47]. Disease recurrence poses a significant burden to patients, diminishing quality of life and increasing morbidity and mortality.

Treatment of recurrent CDI is challenging and there is no uniformly effective therapeutic approach. For the first recurrent episode, the most recent treatment guidance, published by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), recommends continued use of the agent employed to treat the initial infection or the use of either vancomycin or fidaxomicin [39]. In treating a first recurrence of CDI, fidaxomicin has been shown to be associated with reduced rates of subsequent recurrences compared with vancomycin [44]. For multiple recurrences, either vancomycin with a tapered and/or pulsed dosing regimen or fidaxomicin is preferred [39].

Therapeutic options become less clear in cases of complicated CDI (fulminant disease that is refractory to antibiotic therapy and may progress to toxic megacolon, bowel perforation or systemic toxicity); total abdominal colectomy with ileostomy may be necessitated [39]. Diverting loop ileostomy combined with intracolonic and intravenous antibiotic therapy is a less invasive alternative to total colectomy and is currently in clinical trials for the treatment of complicated CDI [39,48].

Faecal microbiota transplantation (FMT) has emerged as an alternative therapy for multiple recurrent CDI. In FMT, healthy donor faeces are introduced into the gut of patients with recurrent CDI. FMT is thought to work by restoring the diversity of the intestinal microbiota, which is essential for colonisation resistance [49,50]. Systematic reviews of FMT have demonstrated the success of this therapy, with cure rates of ca. 90% [51,52]. The first randomised controlled trial (RCT) of FMT, which was a small, open-label study, demonstrated the efficacy of oral vancomycin combined with nasoduodenal infusion of donor faeces over vancomycin therapy alone (81% vs. 23–31% clinical cure, respectively) for the treatment of multiple recurrent CDI [53]. In addition, FMT has shown efficacy in cases of fulminant CDI [54]. FMT is a promising alternative therapy for multiple recurrent CDI and it is now recommended, in combination with oral vancomycin, for the treatment of multiple recurrent CDI [39]; however, the long-term safety of the procedure remains unclear, especially as more is learnt regarding the far-reaching effects of the human gut microbiome.

In addition to FMT, the oral microbiome therapeutics SER-109 and RBX2660 appear to be promising bacteriotherapies for CDI [55,56].

2. Ridinilazole (formerly SMT19969)

Ridinilazole [2,2′-bis(4-pyridyl)3H,3′H 5,5′-bibenzimidazole] is a novel antibacterial that does not appear to act through the classical pathways associated with antibiotics, such as inhibition of cell wall, protein, lipid, RNA or DNA synthesis. In fluorescent-labelling studies, treatment of *C. difficile* with antibiotic concentrations below the minimum inhibitory concentration (sub-MICs) of ridinilazole resulted in a filamentous phenotype with replicated nucleoids along the length of the cell and no observed septum formation, indicating that ridinilazole may impair cell division [57]. Ridinilazole is being developed by Summit Therapeutics plc (Abingdon, UK).

2.1. In vitro efficacy of ridinilazole

2.1.1. Minimum inhibitory concentration assays

In susceptibility testing of 82 clinical isolates of *C. difficile* (including ribotype 027), ridinilazole displayed potent growth inhibition and had lower MICs [MIC range, 0.06–0.25 μg/mL; MIC for 90% of the organisms (MIC_{90}), 0.125 μg/mL] than metronidazole (MIC range, 0.125–8 μg/mL; MIC_{90}, 8 μg/mL) or vancomycin (MIC range, 0.5–4 μg/mL; MIC_{90}, 2 μg/mL) [58,59]. Similarly, ridinilazole was found to be more potent than metronidazole or vancomycin at inhibiting the growth of 50 ribotype-defined *C. difficile* strains [60].

Ridinilazole-induced growth inhibition was also assessed in a recent study of 107 *C. difficile* clinical isolates covering a range of defined resistance phenotypes (e.g. resistance to antibiotics

Table 1
In vitro minimum inhibitory concentrations (MICs) of ridinilazole, fidaxomicin, vancomycin and metronidazole for distinct ribotypes of *Clostridium difficile*.

Ribotype/Drug	MIC (μg/mL)			Reference
	Range	MIC_{50}	MIC_{90}	
Ribotype 001 (n = 10)				
Ridinilazole	0.06–0.125	0.125	0.125	[58]
Fidaxomicin	0.008–0.06	0.03	0.06	
Vancomycin	0.5–4	1	4	
Metronidazole	0.125–1	1	1	
Ribotype 002 (n = 8)				
Ridinilazole	0.125–0.25	0.25	NR	[60]
Fidaxomicin	0.06–0.25	0.25	NR	
Vancomycin	1–2	1	NR	
Metronidazole	0.25–0.5	0.5	NR	
Ribotype 005 (n = 3)				
Ridinilazole	0.25	0.25	NR	[60]
Fidaxomicin	0.06–0.25	0.25	NR	
Vancomycin	2	2	NR	
Metronidazole	0.5	0.5	NR	
Ribotype 014 (n = 8)				
Ridinilazole	0.125–0.25	0.125	NR	[60]
Fidaxomicin	0.06–0.5	0.25	NR	
Vancomycin	1–2	1	NR	
Metronidazole	0.25–0.5	0.5	NR	
Ribotype 027 (n = 11 [58]; n = 11 [60])				
Ridinilazole	0.25–0.5	0.25	0.25	[60]
	0.125–0.25	0.125	0.125	[58]
Fidaxomicin	0.5–1	0.5	0.5	[60]
	0.03–0.06	0.06	0.06	[58]
Vancomycin	1–8	2	4	[60]
	0.5–4	1	2	[58]
Metronidazole	2–8	2	8	[60]
	1–2	2	2	[58]
Ribotype 054 (n = 4)				
Ridinilazole	0.125–0.25	0.25	NR	[60]
Fidaxomicin	0.125	0.125	NR	
Vancomycin	1–2	1	NR	
Metronidazole	0.5	0.5	NR	
Ribotype 106 (n = 10 [58]; n = 3 [60])				
Ridinilazole	0.25	0.25	NR	[60]
	0.125–0.25	0.125	0.125	[58]
Fidaxomicin	0.5	0.5	NR	[60]
	0.03–0.125	0.06	0.125	[58]
Vancomycin	1	1	NR	[60]
	0.5–4	1	2	[58]
Metronidazole	0.5	0.5	NR	[60]
	1–2	2	2	[58]

$MIC_{50/90}$, MIC for 50% and 90% of the organisms, respectively; NR, not reported.

commonly associated with CDI) [61]. In this study, all isolates were susceptible to ridinilazole, and cross-resistance was not observed.

The activity of ridinilazole against specific *C. difficile* ribotypes (including ribotypes 001, 002, 005, 014, 027, 054 and 106) was similar, with an MIC range of 0.06–0.5 μg/mL and an MIC_{90} of 0.125 μg/mL (Table 1). These data demonstrate that there are no major differences between *C. difficile* ribotypes in terms of susceptibility to ridinilazole. In addition, ridinilazole was more active against 11 ribotype 027 strains than either metronidazole or vancomycin (Table 1).

Antibiotics for CDI (both marketed and in development) typically achieve gastrointestinal concentrations that are significantly in excess of the agent's MIC. Metronidazole is an exception, as it is highly absorbed following oral administration resulting in low intraluminal drug concentrations [62]. Isolates showing reduced susceptibility to metronidazole that remain susceptible to other agents, including ridinilazole, have been reported [63], and whilst no link between clinical outcome, intraluminal concentration and the MIC of metronidazole has been established, elevated MICs may be a future consideration for metronidazole.

A comparative study of 174 Gram-positive and 136 Gram-negative intestinal anaerobes showed both ridinilazole and fidaxomicin to be inactive (MIC_{90} > 512 μg/mL) against *Bacteroides* spp. (including *Bacteroides fragilis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron* and *Bacteroides vulgatus*) [60]. Whereas ridinilazole was relatively inactive against these species, metronidazole and vancomycin MICs were generally in the ranges of 0.5–2 μg/mL and 8–256 μg/mL, respectively [60].

In general, ridinilazole and fidaxomicin display limited activity against Gram-negative anaerobes, which suggests that these drugs could potentially spare the normal intestinal microbiota. Whereas fidaxomicin showed activity against a number of Gram-positive anaerobes, such as *Bifidobacterium* spp. and *Eggerthella* spp., ridinilazole showed limited activity against these species with MIC_{90} values of >512 μg/mL [60]. Ridinilazole also had limited activity against Gram-positive aerobes, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus* spp. and *Enterococcus faecium* [60].

The activity of ridinilazole against other *Clostridium* spp. was species-dependent, with MIC_{90} values of >512 μg/mL for *Clostridium perfringens* and *Clostridium ramosum* and 1 μg/mL for *Clostridium innocuum* [60]. Similarly, in a study of 162 strains of 35 less frequently recovered intestinal *Clostridium* spp. in clusters I–XIX, ridinilazole MICs ranged from 0.06 μg/mL to >512 μg/mL, and resistance to ridinilazole was neither cluster- nor species-dependent [64].

2.1.2. *Bactericidal activity of ridinilazole*

Ridinilazole displayed bactericidal activity against *C. difficile* ribotype 027, with all concentrations of ridinilazole resulting in a >3.2 log_{10} reduction in CFU/mL at 24 h [58]. At most concentrations tested, vancomycin was bacteriostatic against ribotype 027, although a 3.1 log_{10} reduction in CFU/mL was observed at 2× MIC at 24 h. Fidaxomicin was bacteriostatic against ribotype 027 at 1–10× MIC and was bactericidal at only 20× MIC (ca. 5- to 50-fold lower than the relative concentrations of drug in the gut) at 24 h. Whereas vancomycin was generally bacteriostatic against *C. difficile* ribotypes 012 and 078, both ridinilazole and fidaxomicin were bactericidal against these strains [58].

2.1.3. *Post-antibiotic effect (PAE) of ridinilazole*

Ridinilazole exhibited a prolonged PAE (4–20 h) against *C. difficile* ribotypes 012, 027 and 078 at high concentrations (10× MIC); there was no growth recovery of *C. difficile* strains following 1 h of treatment at 20× MIC [58]. Vancomycin displayed a minimal PAE (0–2 h) at all concentrations tested, whereas fidaxomicin had a prolonged PAE (8–20 h) at ≥2× MIC [58], as previously reported.

2.1.4. *Effect of ridinilazole on* C. difficile *toxin production*

Treatment of *C. difficile* ribotype 027 (R20291) with either supra- (4× and 40×) or sub- (0.5×) MICs of ridinilazole resulted in statistically significant reductions both in toxin A and toxin B concentrations [57]. At 0.5× MIC of ridinilazole, toxin B was not detected and toxin A was decreased by 80–90%. Toxin A and toxin B decreased by 80–90% after 24 h of exposure to ridinilazole at supra-MIC. In addition, treatment of Caco-2 cells with ridinilazole-treated culture supernatants resulted in a marked 74% reduction in IL-8 release compared with treatment with drug-free supernatants. Both vancomycin and metronidazole controls showed similar levels of toxin production and IL-8 release compared with drug-free controls [57].

2.1.5. *Human gut model of* C. difficile *infection*

The narrow spectrum of activity of ridinilazole observed in MIC assays was further supported by an in vitro human gut model of clindamycin-induced CDI. In this clinically predictive model, faecal emulsions are used to establish a steady-state gut microbiota in

pH-maintained fermentation vessels, which are subsequently inoculated with *C. difficile* spores and then clindamycin to establish CDI (as manifested by cytotoxin production) before treatment with the test antimicrobial agent. This model has been used successfully to assess the ability of antibiotics to induce CDI as well as to examine the efficacy of antimicrobial agents for CDI treatment [65–68].

Introduction of ridinilazole into the human gut model spared the normal anaerobic microbiota whilst specifically inhibiting the viability of *Clostridium* spp. (2 $\log_{10}$ reduction in CFU/mL) [59]. Ridinilazole caused a rapid decline in *C. difficile* cytotoxin titres, and toxin remained undetectable in the final days following cessation of ridinilazole treatment. In this study, there was no evidence for recurrent CDI in the gut model following ridinilazole treatment [59].

Taken together, these in vitro data demonstrate the narrow spectrum of activity of ridinilazole, which specifically targets *C. difficile* isolates whilst sparing other intestinal anaerobes and faecal aerobes.

2.2. In vivo efficacy of ridinilazole

2.2.1. Preclinical animal models

The hamster model of clindamycin-induced CDI is the standard in vivo model for CDI. Animals develop large bowel colonisation, *C. difficile* spore formation and, ultimately, fatal toxin-mediated gastrointestinal inflammation. The model includes an acute infection and treatment phase followed, in some cases, by a period to monitor for recurrent disease [69]. Compared with vancomycin, both ridinilazole and fidaxomicin displayed greater efficacies in this model against *C. difficile* ribotype 027 strain [70]. A twice-daily dose of either ridinilazole or fidaxomicin conferred protection from CDI with 90–100% survival of hamsters at Day 28, whereas twice-daily vancomycin resulted in only 10% survival (see Fig. 1) [70]. Plasma levels of ridinilazole were below the limit of detection, demonstrating the low systemic absorption of ridinilazole from the gut [70]. Ridinilazole-treated hamsters were culture-negative for *C. difficile* spores for a longer period than fidaxomicin-treated hamsters, with higher doses of either of these agents inhibiting spore recovery from faecal samples beginning at Day 7 and continuing through the 28-day post-treatment follow-up period [70].

In a hamster model of CDI with a once-daily dosing regimen, ridinilazole displayed greater efficacy than vancomycin both against non-epidemic and epidemic strains of *C. difficile* [71]. Similar to the twice-daily dosing study, plasma levels of ridinilazole were below the level of detection, whereas caecal ridinilazole concentrations were well above the MIC [71], thus demonstrating the non-absorbable nature of ridinilazole and minimal systemic exposure.

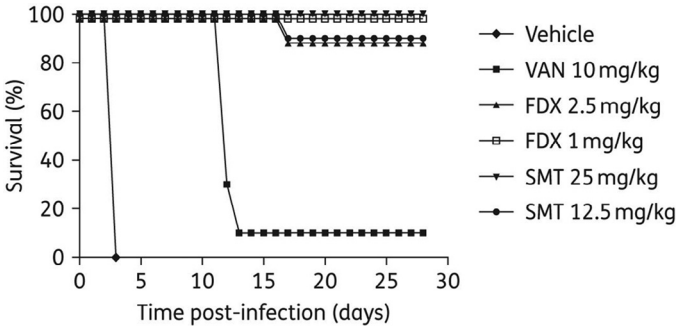

Fig. 1. Survival (%) of hamsters following infection with *Clostridium difficile* BI1 (ribotype 027). VAN, vancomycin; FDX, fidaxomicin; SMT, ridinilazole. Reproduced from Ref. [70] (fig. 1). By permission of Oxford University Press on behalf of The British Society for Antimicrobial Chemotherapy.

2.2.2. Phase 1 safety, tolerability and pharmacokinetics of ridinilazole

The safety, tolerability and pharmacokinetics of ridinilazole were examined in a phase 1, double-blind, placebo-controlled trial in healthy volunteers [72]. Fifty-six male subjects received either a once-daily oral dose of ridinilazole (2, 20, 100, 400, 1000 or 2000 mg) or a twice-daily oral dose of ridinilazole (200 mg or 500 mg) for 9 days with a final dose on Day 10. Ridinilazole was found to be safe and was well tolerated at all doses tested. The incidence of adverse events (AEs) was comparable between subjects receiving ridinilazole or placebo; AEs that were possibly or likely due to ridinilazole were mild and there was no dose-dependent relationship between ridinilazole and the incidence or severity of AEs. No clinically significant findings were observed for blood pressure, body temperature, 12-lead electrocardiogram (ECG), clinical laboratory evaluations, faecal occult blood or physical examination [72].

Oral administration of ridinilazole was associated with negligible systemic exposure. Although plasma ridinilazole concentrations increased following drug administration with food, the maximum observed plasma concentration was 0.305 ng/mL. Low systemic exposure was associated with high gastrointestinal levels of ridinilazole. Following twice-daily dosing at 200 mg, the mean (range) Day 5 and Day 10 faecal concentrations of ridinilazole were 1466 (847–2390) μg/g and 1364 (783–1980) μg/g, respectively [72]. These values were markedly higher than the ridinilazole MIC range for *C. difficile* of 0.06–0.5 μg/mL (Table 1).

The phase 1 study also examined the effect of ridinilazole on gut microbiota (Fig. 2). Culture methods were used to quantify changes in the composition of gut microbiota, including the presence of *Bacteroides*, bifidobacteria, lactobacilli, total clostridia, total anaerobes, lactose-fermenting Enterobacteriaceae and total aerobes. Faecal samples were collected pre-dosing and at the mid and endpoint of the 200 mg and 500 mg twice-daily dosing regimens. Repeat oral administration of ridinilazole caused minimal changes in bacterial counts, except for total clostridia in which a >3 $\log_{10}$ reduction was observed at both doses at the midpoint of dosing; clostridial counts remained below the limit of detection at the end of dosing

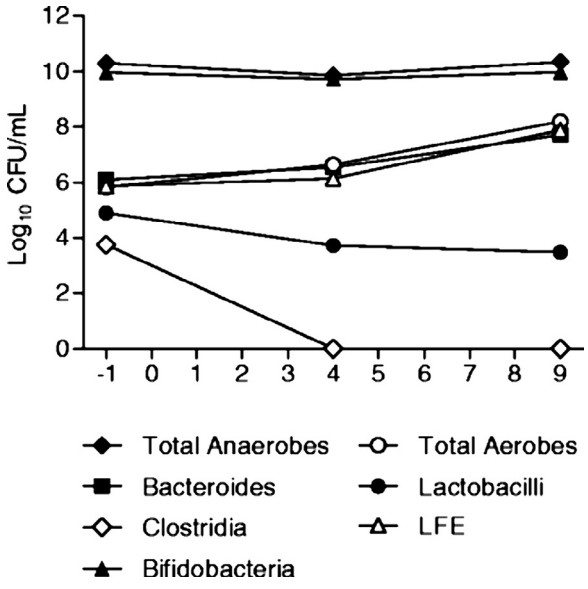

Fig. 2. Change in gut microbiota composition for healthy volunteers in study Group G, which included eight males who received a twice-daily 200 mg oral dose of either ridinilazole ($n = 6$) or placebo ($n = 2$). Microbiota composition, as measured by culture techniques, is represented as mean $\log_{10}$ CFU/mL for the indicated bacteria. LFE, lactose-fermenting Enterobacteriaceae. Reproduced from Ref. [72] (fig. 2). By permission of the original publisher BioMed Central.

[72]. Overall, ridinilazole was associated with negligible changes in counts of other bacterial groups, including *Bacteroides*, bifidobacteria and lactobacilli, which have been associated with being important components of the complex ecology of the healthy bowel microbiota that protects against CDI (termed colonisation resistance) [73,74]. These data indicate that ridinilazole causes minimal damage to the gut microbiota, thus allowing the natural restoration of colonisation resistance during CDI treatment. This lack of collateral damage to the gut microbiota may result in reduced rates of recurrent disease.

3. Discussion

In addition to the high burden that CDI poses to patients (recurring episodes of infection and increased morbidity and mortality), there is also a growing economic burden to hospitals and the healthcare system, particularly in the costs of treating multiple episodes of CDI [75]. Recent studies have estimated yearly costs of CDI at ca. €3 billion in the EU and between US$436 million and US$3.2 billion in the USA [76]. Thus, there exists a significant unmet and increasing medical need for new therapies to treat CDI, specifically those that can reduce the rate of disease recurrence.

Understanding the role that the intestinal microbiome plays in the pathogenesis of CDI is essential to the development of effective therapies for this burdensome, and in some instances fatal, disease. It is known that mere exposure to *C. difficile* spores through the faeco–oral route does not necessarily lead to CDI, as favourable conditions in the host gastrointestinal environment are required for spore germination [77–79]. In addition, a critical function of the normal gut microbiota is to maintain colonisation resistance, by which pathogenic organisms are unable to establish and proliferate in the host gut [49,80].

The intestinal microbiota mediates colonisation resistance through several direct and indirect mechanisms, such as nutrient metabolism, niche exclusion, production of antimicrobial peptides and modulation of the host immune system [49,81–84]. Hence, the microbiota represents a fundamental component of host intestinal physiology, playing central roles in metabolism and immune function.

Antibiotic use in humans has been shown to disrupt the composition and to decrease the diversity of the gut microbiota [85]. This antibiotic-induced microbial imbalance (or dysbiosis) is thought to lead to functional changes in the host intestinal environment that impair colonisation resistance, thereby conferring susceptibility to CDI [80,86]. The risk of developing CDI is significantly higher immediately following or during antibiotic administration; the CDI incidence rate was over two-fold higher with concomitant antibiotic therapy or when patients had received antibiotics in the previous 5 days [87]. In patients with recurrent CDI, there is decreased diversity in the intestinal microbiota compared with either non-CDI patients or cured CDI patients who did not experience a recurrence [88].

Although colonisation resistance has been associated with specific bacterial taxonomic groups, such as *Clostridium* clusters IV and XIVa [89], Clostridiales Incertae Sedis XI [90], butyrogenic bacteria [82], *B. fragilis* [73] and *Bifidobacterium longum* [74], it has recently been shown that different absolute compositions of bacterial communities can protect against CDI and that a defining characteristic of a protective bacterial community is an appropriate level of diversity with a normal functional environment [86,91].

Ideal therapies for CDI would specifically target *C. difficile* whilst leaving the indigenous gut microbiota intact, thereby allowing restoration of colonisation resistance and the healthy microbiome during treatment. Metronidazole and vancomycin currently remain the mainstay antibiotics for treatment of CDI, but these agents have a broad spectrum of activity and cause significant disruption to the normal gut microbiota [92]. The repeated use of these antibiotics for treatment of recurrent CDI promotes dysbiosis, which further impairs colonisation resistance. In addition, metronidazole and vancomycin have been shown to promote the outgrowth of vancomycin-resistant enterococci [93]. Owing to its narrow spectrum of activity, minimal disruption to the normal intestinal microbiota and reduced rates of recurrent disease, fidaxomicin offers advantages over both metronidazole and vancomycin for the treatment of CDI [73,89,94]. Thus far, FMT has been a highly successful therapy for multiple recurrent CDI, presumably due to restoration of a diverse faecal microbiome with intact colonisation resistance [50]. FMT is typically used as a salvage therapy in the most severe cases of CDI, as its efficacy has not been fully validated for the treatment of initial episodes of CDI. Furthermore, its efficacy and long-term safety profile for treating multiple recurrent CDI requires further assessment in RCTs.

Ridinilazole is a novel antibiotic that could potentially fulfil the requirements for improved CDI treatment with its highly targeted spectrum of activity and ability to spare the normal gut microbiota [60,64,72]. Ridinilazole is bactericidal against *C. difficile* and exhibits a prolonged PAE [58] that results in decreased cytotoxin titres and spore counts [59,70]. Hamster models of clindamycin-induced CDI have demonstrated the efficacy of ridinilazole for treating acute CDI and preventing recurrent disease [70,71]. In a phase 1 study, oral ridinilazole was well tolerated, displayed low systemic exposure and specifically targeted clostridia whilst sparing other anaerobic and aerobic faecal bacteria [72]. Because ridinilazole therapy shows negligible disruption to the normal gut microbiota, this agent has the potential to treat CDI whilst significantly reducing the likelihood of disease recurrence.

Further studies in CDI patients are required to validate the efficacy of ridinilazole for CDI treatment, although initial results from the multicentre phase 2 CoDIFy trial of 100 CDI patients have demonstrated superiority of ridinilazole over vancomycin on sustained clinical response (66.7% vs. 42.4%, respectively) [95]. Sustained clinical response was defined as clinical cure at the end of the 10-day treatment period and the absence of recurrence within 30 days of treatment end. These early phase 2 results suggest that ridinilazole may be effective at reducing disease recurrence, a central unmet need in CDI treatment. Phase 3 studies will hopefully shed more light on the clinical utility of ridinilazole in CDI.

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