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Ridinilazole: a novel antimicrobial for *Clostridium difficile* infection

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Abstract

Clostridium difficile (*C. difficile*) infection remains a global healthcare threat worldwide and the limited options available for its treatment are of particular concern. Ridinilazole is one potential future agent, as it demonstrates rapid bactericidal activity against *C. difficile*. Current studies show that ridinilazole has a lower propensity for collateral damage to the gut microbiome and appears to diminish the production of *C. difficile* toxins. Results from phase II studies demonstrate that patients receiving ridinilazole had a higher sustained clinical response compared with patients receiving vancomycin (66.7% vs. 42.4%; P=0.0004). Adverse reactions were similar between ridinilazole and vancomycin (40% vs. 56%, respectively), with most being gastrointestinal-related. Nausea (20%) and abdominal pain (12%) were the most commonly reported adverse reactions associated with ridinilazole. Phase II study results are promising and future availability of phase III trial results will help further delineate the role and value of ridinilazole.

Keywords Ridinilazole, *Clostridium difficile*, infectious diarrhea

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Introduction

Clostridium difficile (*C. difficile*) is recognized as an urgent threat to human health and represents an extremely challenging pathogen, given its impact on the healthcare system [1-5]. *C. difficile* infection (CDI) is recognized as the most common healthcare-associated infection and has spread across the globe [1,6-9]. Efforts in antimicrobial stewardship and infection prevention have demonstrated effectiveness in reducing the burden of CDI [10-13]. Unfortunately, CDI remains a looming presence in modern healthcare. One of the most challenging aspects of managing patients with *C. difficile* is the pathogen's propensity to cause recurrent infections. Recurrence rates of up to 25% have been reported following treatment with metronidazole or vancomycin [14,15]. The vicious cycle of recurrence continues further, with patients who have one recurrence being at increased risk for another [16].

Treatment of acute CDI is currently limited to the antibiotics metronidazole, vancomycin and fidaxomicin, with bezlotoxumab being an option as adjunctive therapy. Metronidazole has largely fallen out of favor because of potential issues of resistance and inferior clinical response [17]. Vancomycin is currently utilized as first-line therapy; however, recurrence remains a substantial issue with this agent [14,17,18]. Fidaxomicin has demonstrated similar efficacy to that of vancomycin with the benefit of decreased recurrence; however, this agent may not be as effective against the fluoroquinolone-resistant BI/NAP1/027 strain (ribotype 027), the predominant ribotype in North America [19-21]. In view of the problematic nature of currently available treatment options, ridinilazole represents a potential welcome addition to the armamentarium. This paper will review the pharmacology, spectrum of activity, pharmacokinetics, pharmacodynamics, clinical trials and safety of ridinilazole.

Data sources

To gather relevant published information, a literature search was performed using PubMed, EMBSCO and Google Scholar electronic databases for relevant publications written

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in the English language. Search terms included ridinilazole, SMT19969, and *C. difficile*. Information was also gathered from abstracts from the Interscience Conference on Antimicrobial Agents and Chemotherapy, IDWeek, American Society for Microbiology Microbe, and the European Congress of Clinical Microbiology and Infectious Diseases.

Chemistry and pharmacology

Ridinilazole, previously known as SMT19969, is a novel antibacterial currently under development for the treatment of *C. difficile*. Its chemical name is 2,2′-bis(4-pyridyl)3H,3′H 5,5′-bibenzimidazole. The agent has a unique mechanism of action and is thought to interfere with cell division. It demonstrates rapid bactericidal activity against *C. difficile* [22,23]. A cluster of *C. difficile* genes involved in cell division were found to display an altered expression following exposure to ridinilazole in a transcriptomic analysis. These findings, combined with previous work demonstrating a filamentous phenotype of *C. difficile* upon exposure to ridinilazole, suggest that the agent works by targeting cell growth [22,24]. Preclinical animal studies demonstrated negligible systemic exposure of the orally administered agent [25]. Following oral administration of 200 mg b.i.d., ridinilazole is minimally absorbed from the gastrointestinal lumen, resulting in low systemic levels and high fecal concentrations [26]. The specificity of activity targeting *C. difficile* and limiting collateral damage to the gut microbiota, along with its minimal systemic absorption, make ridinilazole an exciting agent for treating CDI.

Microbiologic activity

Activity against *C. difficile*

Ridinilazole has demonstrated potent activity against a breadth of *C. difficile* strains *in vitro* [27-32]. In a study of 107 *C. difficile* strains of varying resistance phenotypes, ridinilazole demonstrated minimum inhibitory concentration (MIC) values (MIC range: 0.015-0.5 mg/L; MIC_{50}: 0.03 mg/L; MIC_{90}: 0.125 mg/L) lower than those of metronidazole (MIC range: <0.125-2 mg/L; MIC_{50}: 0.5 mg/L; MIC_{90}: 2 mg/L) and vancomycin (MIC range: 0.5-8 mg/L; MIC_{50}: 1 mg/L; MIC_{90}: 2 mg/L), and comparable to those of fidaxomicin (MIC range: 0.004-0.125 mg/L; MIC_{50}: 0.06 mg/L; MIC_{90}: 0.125 mg/L) [29]. Among 200 isolates collected from *C. difficile* toxin-positive stool samples at 6 United States sites, ridinilazole was similarly found to have lower MIC values (MIC range: 0.12-0.5 mg/L; MIC_{50}: 0.12 mg/L; MIC_{90}: 0.25 mg/L) than metronidazole (MIC range: 0.12-2 mg/L; MIC_{50}: 0.25 mg/L; MIC_{90}: 1 mg/L) or vancomycin (MIC range: 0.25-4 mg/L; MIC_{50}: 1 mg/L; MIC_{90}: 2 mg/L) and was comparable to fidaxomicin (MIC range: 0.015-1 mg/L; MIC_{50}: 0.03 mg/L; MIC_{90}: 0.125 mg/L) [30].

Mutant prevention concentrations, used to describe the antimicrobial drug concentration required to block the growth of the least susceptible cell present in a high-density bacterial population and give insight into the likelihood for resistance selection compared with achievable therapeutic concentrations, remained well below (≤0.25 mg/L) fecal concentrations of ridinilazole, suggesting a low propensity for resistance selection among *C. difficile* strains clinically [33]. Following 14 serial passages at sub-MIC concentrations (0.5 × MIC), no increase in ridinilazole MIC was noted and spontaneous mutations were highly infrequent, occurring at $<3.17 \times 10^{-9}$ [34]. Moreover, no spontaneously ridinilazole-resistant mutants were identified among 2 clinical strains of *C. difficile* (ribotype 012 and ribotype 027) following 15 serial passages using brain heart infusion broth containing ridinilazole concentrations ranging from 0.004-64 mg/L [35]. In contrast, Bassères *et al* were able to isolate a stable mutant of *C. difficile* ribotype 027 with a filamentous phenotype following 11 serial passages that stabilized at an MIC of 0.48 mg/L, eight times higher than the initial value of the isolate (0.06 mg/L) [36]. Overall, the development of *C. difficile* resistance to ridinilazole seems likely to be uncommon.

Activity against gut microbiota

While exhibiting substantial activity against *C. difficile*, ridinilazole is largely specific for this bacterium and demonstrates limited activity against gut microbiota. When tested *in vitro* against 350 bacterial isolates representing common intestinal flora, ridinilazole showed limited activity against gram-negative and gram-positive anaerobes other than *Clostridium* spp., summarized in Table 1. Of note is the demonstrated lack of activity of ridinilazole against *Bacteroides fragilis* (*B. fragilis*) (MIC range: 512 to >512 mg/L; MIC_{50}: >512 mg/L) and *Bifidobacteria* spp. (MIC range: 16 to >512 mg/L; MIC_{50}: >512 mg/L). In contrast, vancomycin MICs for *B. fragilis* and *Bifidobacteria* spp. ranged from 32-128 mg/L and 0.5-1 mg/L, respectively [27]. In a subsequent evaluation of 162 strains representing 35 species of *Clostridium* spp. (non-*C. difficile*), ridinilazole showed variable activity MIC range (0.06 to >512 mg/L) depending upon species and strain [37]. In an *in vitro* CDI human gut model, ridinilazole demonstrated minimal impact on viable cell counts of indigenous gut microbiota other than total *Clostridium* spp. [32].

In fecal samples from healthy adult males who were given ridinilazole 200 or 500 mg b.i.d. in a Phase I trial, clostridial viable counts were below the lower limits of detection for viable counting and other gut microbiota were largely unchanged [38]. Further phase I evaluation similarly showed a limited impact of ridinilazole on gut microbiota other than *Clostridium* spp. Counts of *Bacteroides* spp. increased (2 log by day 9), while counts of lactobacilli decreased (1 log by day 4, remaining constant until day 9). Minimal change was observed in counts of *Bifidobacterium* spp. and total anaerobes, while counts of total aerobes and lactose fermenting *Enterobacteriaceae* increased (2 log by day 9) [26]. An evaluation of fecal samples from 82 patients enrolled in a phase II trial similarly demonstrated a limited impact of ridinilazole on gut microbiota, except for a 1 $\log_{10}$ reduction on day 10 of therapy for *Clostridium leptum*. In

Table 1 *In vitro* susceptibility of various gastrointestinal bacteria to ridinilazole and select comparator agents [27,34]

Ribotype	Agent	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Bifidobacterium species	Ridinilazole	16→512	64, >512	128, >512
	Fidaxomicin	≥0.03-0.25	0.125	0.125
	Vancomycin	0.5-1	1	1
	Metronidazole	2→512	32	128
Bacteroides fragilis	Ridinilazole	512→512	>512	>512
	Fidaxomicin	>512	>512	>512
	Vancomycin	32-128	64	64
	Metronidazole	0.5-2	1	2
Clostridium innocuum	Ridinilazole	0.06-1	0.25	1
	Fidaxomicin	128-512	256	256
	Vancomycin	16	16	16
	Metronidazole	0.5-16	1	2
Clostridium ramosum	Ridinilazole	128→512	>512	>512
	Fidaxomicin	>512	>512	>512
	Vancomycin	4	4	4
	Metronidazole	0.5-8	0.5	1
Enterococcus faecalis	Ridinilazole	128→512	>512	>512
	Fidaxomicin	1-8	8	8
	Vancomycin	1-4	1	4
	Metronidazole	>512	>512	>512
Enterococcus faecium	Ridinilazole	64→512	128	128
	Fidaxomicin	0.5-16	8	>128
	Vancomycin	0.5-256	0.5	256
	Metronidazole	256→512	>512	>512
Lactobacillus species	Ridinilazole	0.06→512	16, 128	>512
	Fidaxomicin	0.25→512	8	>512
	Vancomycin	0.5→512	256	>512
	Metronidazole	2→512	>512	>512
Peptostreptococcus anaerobius	Ridinilazole	0.125-128	64	64
	Fidaxomicin	≥0.03	≥0.03	≥0.03
	Vancomycin	0.5	0.5	0.5
	Metronidazole	0.125-1	0.5	1
Prevotella species	Ridinilazole	32→512	>512	>512
	Fidaxomicin	64→512	>512	>512
	Vancomycin	64→512	128	512
	Metronidazole	0.25-1	0.5	1

MIC, minimum inhibitory concentration

contrast, vancomycin exposure led to significantly decreased bacterial counts of *Bacteroides* spp., *Prevotella* spp., *Clostridium coccoides* and *Clostridium leptum* from day 1 to 10 by approximately 3 $\log_{10}$ copies per gram of stool. This substantial impact led to increased bacterial counts of *Enterobacteriaceae* during vancomycin treatment, but not during ridinilazole treatment [39]. A separate exploratory phase II trial evaluated the impact of ridinilazole (n=14) and fidaxomicin (n=13) on the host gut microbiome in a randomized, open label study. The results suggested that ridinilazole was more preserving of the gut microbiome compared with fidaxomicin. Specifically, fidaxomicin exposure led to a reduction in 10 of the bacterial families analyzed compared to only 2 bacterial families with reduced populations among samples exposed to ridinilazole.

Moreover, fidaxomicin exposure led to a reduction in the population of bacterial families belonging to the Firmicutes, thought to have a role in protection against CDI [40]. An additional phase II trial compared the composition of fecal microbiota between patients administered ridinilazole and vancomycin using 16S rDNA at baseline, day 5 of therapy, day 10 (end of therapy), day 25 and at the end of the study. Significant reductions in percent relative abundance were modest and were only found within the Firmicutes phylum among samples from ridinilazole-exposed patients. Within the Firmicutes, vancomycin affected more taxa than ridinilazole. Additionally, vancomycin exposure led to substantial decreases in the abundance of Actinobacteria, Bacteroidetes, and Proteobacteria. Interestingly the fecal microbiota returned to baseline more quickly among ridinilazole-treated patients [41]. The targeted activity of ridinilazole has demonstrated a lower propensity for collateral damage to the gut microbiome in multiple models and human fecal evaluations and highlights a promising aspect of this new agent.

Pharmacokinetics

A phase I study (n=56) was conducted to elucidate the pharmacokinetics and safety of ridinilazole in healthy volunteers after single and multiple oral doses taken with or without food [26]. All doses studied were well-tolerated and any adverse effects potentially due to ridinilazole were mild. In part one, placebo or single ascending doses of ridinilazole (2, 20, 100, 400, 1000 or 2000 mg) were administered. Plasma levels following doses up to 2000 mg in the fasted state were generally undetectable. The 1000 mg dose was studied in the fasted and fed state. A single oral dose of 1000 mg in the fed state produced low but detectable plasma concentrations in all 6 subjects in that study arm (range: 0.102-0.296 ng/mL). Maximum plasma concentrations were observed 4 h following administration. In part two, ridinilazole was administered in the fed state at a dose of 200 mg or 500 mg b.i.d. for 10 days. By Day 10, low plasma ridinilazole concentrations were observed in most test subjects (range: 0.105-0.305 ng/mL) with T_{max} again occurring at 4 hours post-dose. In those receiving 200 mg b.i.d., the mean area under the plasma concentration-time curve from time zero up to the last quantifiable concentration was 0.670 ng.h/mL (range: 0.524-1.30), with average maximum plasma concentrations of 0.141 ng/mL (range: 0.108-0.243). These findings are consistent with those of prior animal studies and subsequent phase II human data, collectively indicating that ridinilazole is mostly contained within the gastrointestinal tract following oral administration [42-44].

Fecal concentrations of ridinilazole were also measured in the phase I study. Ridinilazole was primarily excreted in the feces as unchanged parent drug, which accounted for 97% of the total peak area. Mean fecal concentrations increased with increasing doses. For subjects receiving 200 mg b.i.d., mean (range) fecal concentrations on Day 5 and Day 10 were 1466 μg/g (847-2390 μg/g) and 1364 μg/g (783-1980 μg/g), respectively [26]. Similar fecal concentrations were observed in a phase II trial, wherein the mean fecal concentration on treatment day 10 was 1373 μg/g [44]. The observed fecal concentrations across clinical and preclinical studies are significantly above the MIC_{90} of ridinilazole for *C. difficile* (0.125-0.25 mg/L) [29,30].

Pharmacodynamics

C. difficile isolates exposed to ridinilazole exhibit elongated morphology. Though exposed cells may continue to replicate DNA initially, septum formation is halted, which ultimately impairs cell division [22]. The pharmacodynamic effects of ridinilazole have been studied *in vitro* against a multitude of *C. difficile* strains. Ridinilazole was tested against 82 clinical *C. difficile* isolates collected in the United Kingdom [28]. The MIC_{90} was 0.125 mg/L, 16- to 32-fold lower than MIC_{90} values for metronidazole and vancomycin. Killing kinetics were compared against fidaxomicin and vancomycin for three different *C. difficile* strains: BI1 (ribotype 027), 630 (ribotype 012) and 5325 (ribotype 078). For the BI1 strain, vancomycin was bactericidal at 2× MIC. Fidaxomicin was bacteriostatic at 1-10× MIC (1.5-2.0 $\log_{10}$ reduction at 24 h) and bactericidal at 20× MIC. Ridinilazole was bactericidal at all concentrations tested, with reduction in viable *C. difficile* counts to below the limit of detection by 24 h. Killing by ridinilazole did not appear dependent on drug concentration. Ridinilazole was bactericidal against strains 630 and 5325 at ≥5× MIC and ≥10× MIC, respectively. The post-antibiotic effect (PAE) for ridinilazole against all strains was >20 h at 20× MIC, a concentration that should be exceeded in patients taking 200 mg by mouth b.i.d. [26,44]. Fidaxomicin also exhibited a PAE for all strains, while vancomycin was generally bacteriostatic with minimal PAE (0-2 h) [28].

Bassères *et al* examined *in vitro* production of toxins A and B in *C. difficile* strains exposed to sub-MIC (0.125×-0.5×) and supra-MIC (4× or 40× MIC) concentrations of ridinilazole [22]. Toxin B production was suppressed below the limit of detection with all concentrations of ridinilazole tested. Toxin A levels were reduced by 75% to >90% at varying ridinilazole concentrations. Cytotoxin levels were similarly reduced in an *in vitro* gut model of CDI [32]. Additionally, interleukin (IL)-8 release from eukaryotic cell lines exposed to antibiotic-treated *C. difficile* isolates were measured [22]. IL-8 production was reduced by 25-74% following exposure to *C. difficile* that had been treated with ridinilazole, suggesting that ridinilazole-mediated toxin reduction may dampen host inflammatory responses. Metronidazole and vancomycin had minimal impact on toxin production, and IL-8 production after exposure to metronidazole or vancomycin was similar to that of controls [22]. In a phase II study, 100 patients with CDI were randomized to 10 days of treatment with either ridinilazole or vancomycin and fecal concentrations of calprotectin and lactoferrin were measured to determine bowel inflammation at baseline, day 5, and day 10 (end of treatment) [45]. When all disease severities were analyzed together at day 10, concentrations of both toxins were reduced similarly from baseline in both the ridinilazole and

vancomycin groups. In contrast, ridinilazole demonstrated a greater reduction in lactoferrin (1.93 vs. 0.62 $\log_{10}$) and calprotectin (1.70 vs. 0.22 $\log_{10}$) from baseline to day 10 when only severe disease was analyzed [45].

The efficacy of ridinilazole versus comparator agents has also been investigated in animal models. The hamster model of clindamycin-induced CDI is a validated method for evaluating the potential of new CDI therapies [46]. In this model, hamsters are rendered susceptible to CDI by oral administration of clindamycin followed by infection with *C. difficile* spores. This protocol produces a severe model of CDI with subject death generally occurring within 3 days in the absence of treatment. In two hamster-model experiments using the epidemic BI/NAP1 (ribotype 025) strain, survival rates with ridinilazole treatment at a total daily dose of 50 mg/kg were 70% and 100% vs. 60% and 10% with vancomycin [42,43]. Fidaxomicin was also effective against the epidemic strain, with 80-90% of animals surviving [42]. In a study arm using the non-epidemic VA11 clinical strain [43], ridinilazole was associated with better survival rates than vancomycin (80-95% vs. 50%). In a study using the non-epidemic ribotype 012 strain [42], all vancomycin-treated animals survived during dosing, but there was a 0% survival rate in the follow-up period. Fidaxomicin conferred some protection, but overall survival rates across different fidaxomicin dosing groups were low, ranging from 0-40%. Ridinilazole conferred significant protection against ribotype 012, with survival rates of 80-100% across dosing groups. Further, following administration of high ridinilazole doses (25 mg/kg b.i.d.), no spores were isolated in animals with ribotype 027 infection from day 7 onwards, although a significant number of animals had spores isolated in the ribotype 012 arm [42].

Safety and efficacy

The safety and efficacy of ridinilazole were compared to those of vancomycin in a phase II, double-blind, active-controlled study at 33 various sites in the United States and Canada [44]. Patients were included in the study if they met the definition of having CDI, had a positive diagnostic test for *C. difficile* or had received more than 24 h of CDI treatment prior to initiation of the study agent. Included patients were randomized 1:1 to receive either ridinilazole 200 mg orally b.i.d. or vancomycin 125 mg orally q.i.d. for 10 days. The primary endpoint was sustained clinical response, which was defined as clinical cure at test of cure (TOC) (day 12-14) and absence of CDI recurrence 30 days after end of treatment (day 10-11). Secondary endpoints and objectives included time to hospital discharge, time to diarrhea resolution, and tolerability of ridinilazole compared with vancomycin. The primary efficacy analysis was performed on 69 patients in the modified intent-to-treat (mITT) population. Patients receiving ridinilazole had a higher sustained clinical response compared with patients receiving vancomycin in the mITT population (66.7% vs. 42.4%; P=0.0004). Ridinilazole was determined to be non-inferior to vancomycin; 36 (77.8%) and 23 (67.7%) patients in the ridinilazole and vancomycin groups, respectively, had a clinical response at TOC. The median time to diarrhea resolution was 4 and 5 days (hazard ratio [HR] 1.19; 90% confidence interval [CI] 0.76-1.87) and median time to hospital discharge was 5 and 7 days (HR 0.99; 90%CI 0.34-2.91) for the ridinilazole and vancomycin groups, respectively. CDI recurrence was documented in 14.3% of patients receiving ridinilazole and 34.8% of patients receiving vancomycin (-16.2%, 90%CI -35.5 to 3).

Ridinilazole displayed adverse reactions similar to those of vancomycin, with most being gastrointestinal-related (40% vs. 56%, respectively) [44]. Three patients had severe adverse reactions that may have been related to the study drug: one patient in the ridinilazole arm had hypokalemia and two patients in the vancomycin arm had serious reactions: one septic shock and moderate hematemesis and the other elevated liver enzymes and diarrhea. Nausea (20%) and abdominal pain (12%) were the most commonly reported adverse reactions associated with ridinilazole. These results were similar to those seen in phase I studies [26].

Concluding remarks

C. difficile infection remains a global healthcare threat with a propensity to cause recurrent disease. Currently, treatment options for *C. difficile* are limited and ridinilazole seems to be a promising potential agent for the treatment of CDI. Ridinilazole has also demonstrated a lower propensity for collateral damage to the gut microbiome and appears to diminish the production of *C. difficile* toxins and subsequent bowel inflammation, which may prove advantageous in managing severe CDI. Current phase II results are promising and future availability of phase III trial results will help further delineate the role and value of ridinilazole.

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