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SMT19969 – A Novel Antibiotic for *C. difficile* Infection
C. difficile Growth Inhibition, Spectrum of Activity and Resistance Development

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Abstract

Background: *C.difficile* infection (CDI) is now established as a major healthcare issue. However, therapy options are limited and recurrent disease remains a significant problem. SMT19969 is the lead compound from a novel class of narrow spectrum, GI restricted, antibiotics in preclinical development for the treatment of CDI. **Methods:** *C. difficile* minimum inhibitory concentrations (MIC) were determined by agar plate dilution on Wilkins Chalgren agar. MIC testing against gut flora bacteria was carried out using CSLI guidelines. Resistance development was evaluated by determining spontaneous mutation frequencies at 4x and 8x MIC and by serial passage over 14 days at sub-inhibitory (0.5 x MIC) drug concentrations **Results:** SMT19969 showed potent *C. difficile* growth inhibition (MICs 0.06-0.25 mg/L; MIC_{90} = 0.125 mg/L) when tested against a panel of 82 clinical isolates comprised of 30 genotypically distinct strains, 31 isolates from ribotypes 027, 106 and 001 and 21 isolates with reduced susceptibility to metronidazole (MICs 4-8 mg/L). SMT19969 showed minimal growth inhibition against a panel of 100 isolates representing members of the gut flora. MIC_{90} values (mg/L) against each group were as follows: Bacteroides spp. >512; *E. coli* and other Proteobacteria > 512; Clostridium spp. other than *C. difficile* = 512; Lactobacillus spp. > 512; Eubacterium spp. = 512; Peptostreptococcus spp. = 128; Bifidobacterium spp. = 128. No drug resistant mutants were isolated. Spontaneous mutation frequencies using the *C. difficile* clinical isolates NCTC13366 and NCTC13307 were <3.17 x10^{-9} and <6.90 x10^{-9} respectively. No increase in SMT19969 MICs for *C. difficile* was seen despite 14 serial passages. **Conclusions:** The high activity and low rate of resistance development in *C. difficile* support the potential use of SMT19969 in the treatment of CDI. The extremely narrow spectrum of activity in gut bacteria testing offers the prospect of reduced risk of CDI recurrence due to flora inhibition.

Introduction

CDI is now established as a major healthcare problem and is a leading cause of morbidity in elderly hospitalised patients. In addition, increasing numbers of cases are being reported in previously low risk groups and there is an increasing awareness of CDI as a community issue. Despite this, therapy options are limited with Vancocin® and Dificid™ being the only FDA approved antibiotics. Metronidazole remains a front-line agent although there are increasing reports of a reduction in efficacy as well as the recent emergence of isolates with reduced susceptibility. Whilst reasonably effective in treating initial infection, the major clinical issue with CDI antibiotics is recurrent disease. Up to 30% of patients receiving vancomycin or metronidazole will experience at least one episode of recurrent CDI with each episode associated with an increased risk of further recurrence and severe disease.[1]

C. difficile outgrowth and toxin production invariably occurs following disruption to the healthy balance of the gut flora, typically as a result of prior antibiotic use. CDI antibiotics, such as vancomycin and metronidazole, continue to suppress significant components of the gut flora during treatment,[2] thereby rendering patients susceptible to recurrent disease. An antibiotic inhibiting *C. difficile* growth whilst allowing gut flora to recover during dosing would be expected to reduce rates of recurrent disease by allowing natural restoration of colonisation resistance. This is supported by the remarkable success (≥ 90% cure rates) of faecal biotherapy which acts to completely restore a healthy ecological balance to the gut flora.[3]

Introduction

SMT19969 is the lead compound from a novel class of antibiotics (**Figure 1**) in preclinical development for the treatment of CDI. The compound shows significantly more potent growth inhibition of *C. difficile* than the current front line agents (vancomycin and metronidazole) and, most importantly, shows an exceptionally narrow spectrum of activity with no significant growth inhibition of Gram positive or Gram negative members of the gut flora (**Table 1** and **2**). This sparing of gut flora is expected to result in a reduction in rates of recurrence by allowing natural restoration of colonisation resistance during treatment.

Methods

Antimicrobial Susceptibility Testing: Minimum inhibitory concentrations (MICs) were determined in duplicate for SMT19969, vancomycin or metronidazole against 82 *C. difficile* clinical isolates by agar dilution on Wilkins Chalgren agar as follows.[4] Isolates were cultured anaerobically overnight in Schaedler's anaerobic broth (0.5 McFarland ~10^7 cfu/mL). One microlitre of culture (~10^4 cfu) was then applied to Wilkins Chalgren agar with or without antimicrobial using a multipoint inoculator. Plates were incubated at 37ºC in an anaerobic atmosphere for 48h, while negative control plates were incubated aerobically at 37ºC. MIC testing against other bacteria and yeasts was carried out using CSLI methodology, [5-8] except Enterococci which were tested anaerobically.[6]

Resistance Development: Spontaneous mutation frequencies were determined at 4 and 8x SMT19969 agar MIC. Cultures were streaked onto multiple SBA plates and grown anaerobically for 48hrs. All of the growth from the plates was harvested and used to prepare a dense cell suspension in 5ml of Brucella Broth. The viable count of this suspension was determined and the cell suspension constituted the inoculum for the study. Duplicate plates were prepared to contain SMT19969 at 4 and 8x the MICs and were each inoculated with 0.1ml of the cell suspension. After 48hrs incubation, the viable count of the inoculum and the number of colonies growing on SMT19969 supplemented plates was determined. Colonies produced were sub-cultured onto SMT19969 agar plates SMT19969 and re-incubated to confirm resistance. The mutation frequency was calculated by dividing the number of confirmed mutants observed by the total number of viable organisms plated. Where no mutant was observed, the mutational frequency was taken as being less than the reciprocal of the total number of viable organisms plated. Selection and amplification of resistance emerging as a result of serial passage (N = 14) at sub-inhibitory concentrations (0.5xMIC) of SMT19969 and metronidazole was determined. Growth from the inoculation at 0.5xMIC was taken, resuspended in Mueller Hinton broth to an inoculum equal to a 0.5 McFarland standard, and the MIC test repeated. This constituted passage 1. This passage was then repeated for a further 13 passages giving 14 passage results in total. Plates showing a change in MIC were stored and isolates suspected of being resistant underwent confirmatory MIC testing by agar dilution.

PLACE HOLDER

Figure 1: Chemical Structure of SMT19969

Results

- SMT19969 showed potent growth inhibition of *C. difficile* (MIC range = 0.06-0.25µg/mL) and no significant differences between SMT19969 MICs against individual *C. difficile* ribotypes was observed (**Table 1**).
  - Overall SMT19969 was significantly more potent than either vancomycin or metronidazole controls with typically 8 fold lower MICs (SMT19969 MIC_{90}=0.125µg/mL; vancomycin, metronidazole MIC_{90}=2-4 µg/mL)
  - The *C. difficile* clinical isolate panel comprised 30 distinct ribotypes covering all major ribotypes of clinical significance including the hyper-virulent ribotype 027 (BI/NAP1), endemic EU ribotypes such as 106 and 001 and emerging strains such as ribotype 078. In addition, isolates with reduced susceptibility to metronidazole were included.
- SMT19969 showed minimal growth inhibition, at therapeutically relevant concentrations, against a range of organisms including key anaerobic and facultative members of the gut flora (**Table 2**).
  - SMT19969 MIC_{90} values against *C. difficile* were typically >1,000 fold lower than those recorded against key members of the gastrointestinal microflora including Gram positive and Gram negative anaerobes such as *Bacteroides spp., Bifidobacterium spp.* and *Lactobacillus spp.* and a range of facultative organisms.
- Resistance development studies demonstrated the low propensity for *C. difficile* to develop SMT19969 resistance.
  - No spontaneous resistant mutants were isolated (mutation frequencies <6.99 x10^{-9} and <3.17 x 10^{-9} **Table 3**) and no increase in SMT19969 *C. difficile* MIC was observed following 14 serial passage at 0.5xMIC (**Figure 2**).
- Additionally, SMT19969 has an ideal drug profile for a CDI antibiotic being orally dosed yet completely retained in the GI tract, shows superior efficacy to vancomycin in the key efficacy models [9] and is extremely well tolerated during *in vivo* toxicology studies.

C. difficile Group (№ isolates)	SMT19969		Metronidazole	Vancomycin
	MIC Range (µg/mL)	MIC_{90} (µg/mL)	MIC_{90} (µg/mL)	MIC_{90} (µg/mL)
Overall Total (82/82)	0.06 - 0.25	0.125	8	2
Genotypically distinct group (30)	0.06 - 0.125	0.125	2	2
Ribotype 001 (10)	0.06 - 0.125	0.125	1	4
Ribotype 027 (11)	0.125 - 0.25	0.125	2	2
Ribotype 106 (10)	0.125 - 0.25	0.125	2	2
Reduced MET susceptibility (21)	0.06 - 0.125	0.125	8	2

Table 1: Minimum Inhibitory Concentration (MIC) for SMT19969,Metronidazole and Vancomycin Against 82 *C. difficile* Clinical Isolates

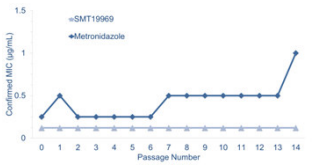

Figure 2: Confirmed MIC for SMT19969 and Metronidazole Following Serial Passage at 0.5xMIC (*C. difficile* NCTC13366)

C. difficile Strain	Mutation Frequency	
	4 x MIC	8 x MIC
NCTC13307	<6.99x10^{-9}	<6.99 x 10^{-9}
NCTC13366	<3.17 x10^{-9}	<3.17 x10^{-9}

Table 3: SMT19969 Spontaneous Mutation Frequencies Against the *C. difficile* Isolates NCTC13307 and 13366

Bacteria (№ Isolates)	MIC range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Bacteroides spp. (16)	128, >512	>512	>512
• *B. fragilis* (10)	>512	>512	>512
• *B. ovatus* (3)	>512	-	-
• *B. caccae* (1)	>512	-	-
• *B. vulgatus* (2)	128	-	-
Lactobacillus spp. (9)	≤0.25 - >512	128	>512
• *L. delbrueckii* (1)	>512	-	-
• *L. casei* (1)	>512	-	-
• *L. paracasei* (1)	>512	-	-
• *L. rhamnosus* (1)	>512	-	-
• *L. acidophilus* (5)	≤0.25 - >512	-	-
Peptostreptococcus spp. (12)	64 - 256	64	128
• *P. anaerobius* (10)	64, 128	-	-
• *P. magnus* (2)	64, 256	-	-
Bifidobacterium spp. (7)	16 - >512	64	128
• *Bifidobacterium spp.* (4)	64 - >512	-	-
• *B. dentium* (1)	64	-	-
• *B. adolescentis* (2)	16, 64		
Clostridium spp. (8)	≤0.25 - >512	16	512
• *Clostridium spp.*(1)	>512	-	-
• *C. tertium* (1)	512	-	-
• *C. sordellii* (1)	256	-	-
• *C. ramosum* (2)	16	-	-
• *C. perfringens* (1)	≤0.25	-	-
• *C. histolyticum* (1)	≤0.25	-	-
• *C. glycolicum* (1)	≤0.25	-	-

Bacteria (№ Isolates)	MIC range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Proteobacteria (21)	>512	>512	>512
• *E. coli* (7)	>512	-	-
• *K. aerogenes* (2)	>512	-	-
• *E. cloacae* +/- ESBL (2)	>512	-	-
• *P. mirabilis* (2)	>512	-	-
• *P. aeruginosa* (2)	>512	-	-
• *S. marcescens* (2)	>512	-	-
• *S. typhimurium* (4)	>512	-	-
Enterococcus spp. (4)	>512	-	-
• *E. faecium* (2)	>512	-	-
• *E. faecalis* (2)	>512	-	-
Fusobacterium spp. (19)	≤0.25, >512	≤0.25	≤0.25
• *F. necleatum* (2)	≤0.25	-	-
• *F. necrogenes* (2)	≤0.25, >512	-	-
• *F. necrophorum* (4)	≤0.25	-	-
• *F. nucleatum* (10)	≤0.25	-	-
• *F. varium* (1)	≤0.25	-	-
Veillonella spp. (2)	≤0.25, >512	-	-
• *V. atypica* (1)	>512	-	-
• *V. parvula* (1)	≤0.25	-	-
Eggerthella spp. (5)	256, 512	256	512
• *E. lenta* (5)	256, 512	-	-
Yeasts (4)	>256	-	-
• *C. albicans* (1)	>256	-	-
• *C. parapsilosis* (1)	>256	-	-
• *S. cerevisiae* (2)	>256	-	-

Table 2: Minimum Inhibitory Concentration (MIC) for SMT19969 Against Anaerobic and Facultative, Gram positive and Gram negative Organisms Representative of the Gut Flora

Conclusions

These data demonstrate the ideal microbiological profile of SMT19969 as a potential front line antibiotic for CDI.

- Potent growth inhibition of *C. difficile*
- Narrow spectrum of activity
- Low propensity to resistance development

The extremely narrow spectrum of activity offers the prospect of reduced rates of recurrent CDI by allowing colonisation resistance to be re-established during the course of dosing.

Preclinical studies are on-going and an IND/CTA filing is expected in H2 2012.

Acknowledgements

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