Comparative In Vitro Activities of SMT19969, a New Antimicrobial Agent, against Clostridium difficile and 350 Gram-Positive and Gram-Negative Aerobic and Anaerobic Intestinal Flora Isolates

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The comparative in vitro activity of SMT19969, a novel, narrow-spectrum, nonabsorbable agent, was studied against 50 ribotype-defined Clostridium difficile strains, 174 Gram-positive and 136 Gram-negative intestinal anaerobes, and 40 Gram-positive aerobic bacteria. SMT19969 was one dilution more active against C. difficile isolates (MIC range, 0.125 to 0.5 μg/ml; MIC90, 0.25 μg/ml), including ribotype 027 strains, than fidaxomicin (range, 0.06 to 1 μg/ml; MIC90, 0.5 μg/ml) and two to six dilutions lower than either vancomycin or metronidazole. SMT19969 and fidaxomicin were generally less active against Gram-negative anaerobes, especially the Bacteroides fragilis group species, than vancomycin and metronidazole, suggesting that SMT19969 has a lesser impact on the normal intestinal microbiota that maintain colonization resistance. SMT19969 showed limited activity against other Gram-positive anaerobes, including Bifidobacteria species, Eggerthella lenta, Finegoldia magna, and Peptostreptococcus anaerobius, with MIC90s of >512, >512, 64, and 64 μg/ml, respectively. Clostridium species showed various levels of susceptibility, with C. innocuum being susceptible (MIC90, 1 μg/ml) and C. ramosum and C. perfringens being nonsusceptible (MIC90 >512 μg/ml). Activity against Lactobacillus spp. (range, 0.06 to >512 μg/ml; MIC90 >512 μg/ml) was comparable to that of fidaxomicin and varied by species and strain. Gram-positive aerobic cocci (Staphylococcus aureus, Enterococcus faecalis, E. faecium, and streptococci) showed high SMT19969 MIC90 values (128 to >512 μg/ml).

MATERIALS AND METHODS

Bacterial strains. The C. difficile isolates were from toxin-positive fecal samples of patients with CDI obtained in 2012 and were ribotyped as previously described (12). The other organisms were recovered from recent clinical samples (2009 to 2011) from humans, mostly from intra-abdominal sources, with some Gram-positive cocci coming from skin and soft-tissue infections. Isolates were identified by standard methods (13, 14) and stored in 20% skim milk at −70°C. They were taken from the freezer and transferred at least twice on supplemented Brucella agar for anaerobes and on Trypticase soy agar for aerobes to ensure purity and growth. Anaerobes were incubated for 48 h and aerobes for 24 h prior to testing. Inocula were prepared by direct suspensions of cells into Brucella broth to achieve the turbidity of the 0.5 McFarland standard. Inocula for aerobes were diluted 1:10 prior to inoculating the plates.

Drugs tested. SMT19969 was obtained from the manufacturer (Summit Corp. PLC, Abingdon, United Kingdom) and reconstituted according to the manufacturer’s instructions by suspending and diluting in dimethyl sulfoxide.
### TABLE 1 Comparative in vitro activity of SMT 19969, fidaxomicin, and metronidazole against anaerobic and aerobic gut bacteria

| Organism (n) and antimicrobial agent | MIC (µg/ml) | Range | MIC₅₀ | MIC₉₀ |
|-------------------------------------|------------|-------|-------|-------|
| **Anaerobic**                        |            |       |       |       |
| **Gram-negative**                    |            |       |       |       |
| *Clostridium difficile* (50)         |            |       |       |       |
| SMT19969                             | 0.125–0.5  | 0.25  | 0.25  |       |
| Fidaxomicin                          | 0.06–1     | 0.25  | 0.5   |       |
| Vancomycin                           | 1–8        | 1     | 4     |       |
| Metronidazole                        | 0.25–8     | 0.5   | 2     |       |
| *Clostridium innocuum* (10)          |            |       |       |       |
| SMT19969                             | 0.06–1     | 0.25  | 1     |       |
| Fidaxomicin                          | 128–512    | 256   | 256   |       |
| Vancomycin                           | 16         | 16    | 16    |       |
| Metronidazole                        | 0.5–16     | 1     | 2     |       |
| *Clostridium perfringens* (11)       |            |       |       |       |
| SMT19969                             | 1–>512     | >512  | >512  |       |
| Fidaxomicin                          | =0.03–0.06 | =0.03 | 0.06  |       |
| Vancomycin                           | 1          | 1     | 1     |       |
| Metronidazole                        | 0.5–4      | 2     | 4     |       |
| *Clostridium ramosum* (10)           |            |       |       |       |
| SMT19969                             | 128–>512   | >512  | >512  |       |
| Fidaxomicin                          | >512       | >512  | >512  |       |
| Vancomycin                           | 4          | 4     | 4     |       |
| Metronidazole                        | 0.5–8      | 0.5   | 1     |       |
| **Bifidobacterium species** (20)     |            |       |       |       |
| SMT19969                             | 16–>512    | >512  | >512  |       |
| Fidaxomicin                          | =0.03–0.25 | 0.125 | 0.125 |       |
| Vancomycin                           | 0.5–1      | 1     | 1     |       |
| Metronidazole                        | 2–>512     | 32    | 128   |       |
| **Lactobacillus species** (20)       |            |       |       |       |
| SMT19969                             | 0.06–>512  | 16    | >512  |       |
| Fidaxomicin                          | 0.25–512   | 8     | >512  |       |
| Vancomycin                           | 0.5–>512   | 256   | >512  |       |
| Metronidazole                        | 2–>512     | >512  | >512  |       |
| **Eggerthella lenta** (20)           |            |       |       |       |
| SMT19969                             | >512       | >512  | >512  |       |
| Fidaxomicin                          | =0.03–0.32 | =0.03 | =0.03 |       |
| Vancomycin                           | 1–8        | 2     | 4     |       |
| Metronidazole                        | 0.125–0.5  | 0.25  | 0.5   |       |
| **Other Gram-positive rod species** (23) | | | | |
| SMT19969                             | 0.06–>512  | 16    | >512  |       |
| Fidaxomicin                          | 0.25–256   | 2     | 128   |       |
| Vancomycin                           | 0.25–4     | 2     | 4     |       |
| Metronidazole                        | 0.25–>32   | 0.5   | 2     |       |
| **Finnigoldia magna** (20)           |            |       |       |       |
| SMT19969                             | 0.03–512   | 1     | 64    |       |
| Fidaxomicin                          | 0.25–32    | 1     | 2     |       |
| Vancomycin                           | 0.25–1     | 0.5   | 0.5   |       |
| Metronidazole                        | 0.125–1    | 0.5   | 1     |       |
| **Parvimonas micra** (20)            |            |       |       |       |
| SMT19969                             | =0.015–0.5 | 0.125 | 0.25  |       |
| Fidaxomicin                          | =0.03–0.06 | =0.03 | =0.03 |       |
| Vancomycin                           | 0.25–1     | 1     | 1     |       |
| Metronidazole                        | =0.03–1    | 0.25  | 0.5   |       |
| **Peptostreptococcus anaerobius** (20)|         |       |       |       |
| SMT19969                             | 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     |       |

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TABLE 1 (Continued)  

Organism (n) and antimicrobial agent | MIC (µg/ml) | Range | MIC₅₀ | MIC₉₀
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**Enterococcus faecalis (10)**  
SMT19969 | 128–512 | >512 | >512 | >512
Fidaxomicin | 1–8 | 8 | 8 | 8
Vancomycin | 1–4 | 1 | 4 | 4
Metronidazole | >512 | >512 | >512 | >512

**Enterococcus faecium (10)**  
SMT19969 | 64–512 | 128 | 128 | 128
Fidaxomicin | 0.5–16 | 8 | >128 | >128
Vancomycin | 0.5–256 | 0.5 | 256 | 256
Metronidazole | 256–512 | >512 | >512 | >512

**Streptococcus species**

| Organism (n) | MIC (µg/ml) | Range | MIC₅₀ | MIC₉₀ |
| --- | --- | --- | --- | --- |

**S. pneumoniae (10)**  
Fidaxomicin | 0.25–0.5 | 0.25 | 0.25 | 0.25
Vancomycin | 0.25 | 0.25 | 0.25 | 0.25
Metronidazole | 0.25–0.5 | 0.25 | 0.25 | 0.25

**Table 2** In vitro activity of SMT19969 against isolates of *Clostridium difficile* according to grouping of ribotypes most frequently encountered in our study

| Ribotype (n) | MIC (µg/ml) | Range | MIC₅₀ | MIC₉₀ |
| --- | --- | --- | --- | --- |

**Table 3** MICs of other ribotypes

| Ribotype no. | SMT19969 | Fidaxomicin | Vancomycin | Metronidazole |
| --- | --- | --- | --- | --- |

**RESULTS**

Results for all organisms other than *C. difficile* are summarized in Table 1. MICs for various *C difficile* strains, categorized by ribotype, are shown in Tables 2 and 3.

Overall, SMT19969 showed potent growth inhibition of the *C difficile* isolates tested, with MIC values one or more dilutions lower than those of fidaxomicin for 42% (21/50) of strains. On a

sulfoxide (DMSO). Since the higher concentrations (64 to 512 µg/ml) of SMT19969 were not soluble in water or molten agar, the final concentrations of DMSO in these plates were as high as 5%. Drug-free control plates containing 5% DMSO were included. All test organisms grew in the presence of 5% DMSO. Comparator drugs were metronidazole, vancomycin (Sigma, St. Louis, MO), and fidaxomicin (Optimer Pharmaceuticals, San Diego, CA) and were reconstituted according to the manufacturers’ instructions.

For anaerobes, MICs were determined using the agar dilution method according to CLSI guidelines (M11-A8) (15) and for facultative and aerobic organisms by the agar dilution method as described in CLSI M7-A9 (16). For anaerobic organisms, supplemented Brucella agar deeps were obtained from Anaerobe Systems (Morgan Hill, CA). Defibrinated sheep blood (Hema Resources Inc., Aurora, OR) was frozen and thawed to obtain the inoculum, the plates were examined for growth and the MICs interpreted (7). Mueller-Hinton agar was used for the aerobic MICs. Medium for streptococci was supplemented with 5% sheep blood. Metronidazole-containing plates were incubated in the anaerobic chamber. SMT19969, metronidazole, vancomycin, and fidaxomicin were tested at concentrations of 0.03 to 512 µg/ml. Quality control strains included *Bacillus fragilis* ATCC 25285, *C. difficile* ATCC 700057, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212. The MIC was defined as the lowest concentration that yielded no visible growth or a marked change/reduction in growth compared to the growth controls (see photos in reference 15 for examples).
weight basis, SMT19969 MICs were equal to those of fidaxomicin for 18 strains, one dilution lower for 17 strains, two dilutions lower for 4 strains, one dilution higher for 8 strains, and two dilutions higher for three strains of *C. difficile*. When analyzed by ribotype, ribotype 027 MICs for SMT19969 were generally one dilution lower than those of fidaxomicin and two to six dilutions lower than those of either vancomycin or metronidazole. Both vancomycin and metronidazole had lower MICs against ribotypes 002 and 014 than ribotype 027, while SMT19969 and fidaxomicin maintained their superior activity against both ribotypes 002 and 014. There were too few isolates in the other ribotype groupings to generalize, although fidaxomicin MICs were one dilution lower than those of SMT19969 against three of the four ribotype 054 strains tested.

Both SMT19969 and fidaxomicin generally were 4- to 16-fold more active than vancomycin and 2- to 8-fold more active than metronidazole against *C. difficile* strains. The prior study (9) which compared SMT19969, metronidazole, and vancomycin against 82 *C. difficile* clinical isolates also used the agar dilution method but with Wilkins-Chalgren agar and ~10^4 CFU/spot inoculum, which is 1 log_{10} less than the inoculum of the CLSI procedure. For their total of 82 strains, including 10 of ribotype 027, they noted SMT19969 to have an MIC range of 0.06 to 0.125 μg/ml with a MIC_{90} of 0.125 μg/ml, which is slightly lower than our results for both general strains and ribotype 027 strains, which may be attributable to differences in media and inoculum concentration. They did note SMT19969 to be more active than either vancomycin or metronidazole in ranges similar to those of our findings.

DISCUSSION

Louie et al. (7) speculated that “retention of components of the normal microflora,” especially *B. fragilis* group species, would have a lesser ecological impact and “might lower the risk of recurrent disease.” Regarding preservation of the normal anaerobic fecal flora components, both SMT19969 and fidaxomicin were inactive against all 60 Bacteroides species (*B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*) and Parabacteroides species isolates, with MICs of >512 μg/ml. Vickers et al. (9) tested 16 Bacteroides species isolates against SMT19969 and found that all isolates had MICs of >512 μg/ml, except for two strains of *B. vulgatus* with MICs of 128 μg/ml. Against other Gram-negative anaerobes, SMT19969 showed minimal activity against Veillonella, Prevotella, and Fusobacterium species other than *F. nucleatum*, with MIC_{90} of >512 μg/ml. *F. nucleatum* isolates were more susceptible to SMT19969 than other Fusobacterium species, with a MIC_{90} of 64 μg/ml (range, 4 to 64 μg/ml). As with vancomycin, Porphyromonas species were highly susceptible to SMT19969, with a MIC_{90} of 0.5 μg/ml (range, 0.015 to 0.5 μg/ml). Similar to fidaxomicin, the relatively poor activity against Gram-negative anaerobes suggests that SMT19969 has a lesser impact on the normal gut microbiota that maintain colonization resistance (7).

Data for Gram-positive anaerobic bacteria showed that SMT19969 was significantly more selective in its activity than fidaxomicin. Minimal growth inhibition was observed against bifidobacteria, Eggerthella lenta, Fingoldia magna, and Peptostreptococcus anaerobius, with SMT19969 MIC_{90} values of >512, >512, 64, and 64 μg/ml, respectively, compared to MIC_{90} values for fidaxomicin of 0.125, ≤0.03, 2, and ≤0.03 μg/ml. The activity of both agents tested against various lactobacilli showed comparable MIC_{90}s and MIC ranges, with susceptibility varying by strain. SMT19969 MIC values against Lactobacillus paracasei and *L. rhamnosus* were >512 μg/ml, whereas other Lactobacillus species showed various levels of susceptibility, ranging from 0.06 to 16 μg/ml.

Against *Clostridium* species, growth inhibition by SMT19969 was species dependent, with MIC_{90}s of >512 μg/ml recorded against *C. perfringens* and *C. ramosum*, whereas *Clostridium innocuum* showed high susceptibility to SMT19969 compared to fidaxomicin, with MIC_{90}s of 1 and 256 μg/ml, respectively.

All the aerobes tested (*S. aureus*, *E. faecalis*, *E. faecium*, and streptococci) showed much higher SMT19969 MICs (128 to >512 μg/ml) than fidaxomicin (4 to 16 μg/ml for *S. aureus*, *E. faecalis*, and *E. faecium* and 16 to 128 μg/ml for streptococci).

Overall, SMT19969 had enhanced activity against *C. difficile* isolates, regardless of ribotype, compared to the other agents tested. It was generally less active than fidaxomicin, vancomycin, and metronidazole against the other Gram-positive aerobes and anaerobes tested. With this relatively narrow spectrum of activity, SMT19969 is likely to have less activity against endogenous gut organisms; thus, it shows promise as a new drug for treating CDI.

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