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Efficacy of SMT19969 and SMT21829 in a Hamster Model of *Clostridium difficile* Associated Disease (CDAD)

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Abstract

Background: *Clostridium difficile* (Cdif) is an important cause of hospital-acquired infectious diarrhea, ranging from a mild self-limiting disease to severe, life-threatening pseudomembranous colitis. SMT19969 and SMT21829 are lead compounds from a novel class of narrow spectrum, GI restricted, antibiotics in preclinical development for the treatment of CDAD. The current study evaluated the efficacy of the SMT compounds and vancomycin (vanco) in the hamster model of CDAD. **Methods:** Male Syrian hamsters were infected by oral gavage with 10^5 CFU of either a non-epidemic or a BI/NAP1 clinical isolate. At 24 hrs post-infection, all animals received a single 10 mg/kg injection of clindamycin. SMT compounds (10, 20, 50 mg/kg) and vanco (20 mg/kg) were administered orally starting 48 hrs after infection and continued once daily for 5 days with survival monitored for 21 days. Cecum samples were tested from all animals for the presence of Cdif toxins A and B. **Results:** MICs against the non-epidemic and BI/NAP1 isolates ranged from 0.125 – 0.25 ug/mL for the SMT compounds and 0.5 – 1 ug/mL for vanco. Against the non-epidemic strain infection, SMT21829 exhibited 100% survival at all doses through day 21 and SMT19969 administration resulted in 80 – 100% survival through day 21 over the dose range tested. Vanco administration resulted in 40 – 60% survival by day 21. Peak cecum levels of SMT19969 reached 172 ug/mL at 3 hrs after a single 20 mg/kg oral dose. For the BI/NAP1 infection, SMT19969 at 20 mg/kg exhibited complete protection out to day 12 (100% survival) with 60% of the animals surviving by day 15 through 21. Vanco at 20 mg/kg also demonstrated 60% survival by the end of the study, but relapse initiated earlier at day 11. **Conclusion:** SMT19969 and SMT21829 exhibited greater efficacy than vanco against both the epidemic BI/NAP1 and non-epidemic isolates evidenced by lower relapse or delayed time to relapse. Efficacy in the hamster CDAD model, coupled with their antimicrobial profile, makes the SMT compounds excellent candidates for further testing as agents for *Clostridium difficile* associated disease.

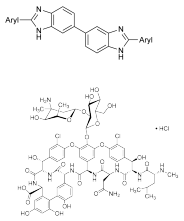
Introduction

Clostridium difficile is an important cause of hospital-acquired infectious diarrhea. Treatment with antimicrobials is the primary risk factor contributing to the development of *C. difficile* diarrheal disease, which ranges from a mild self-limiting disease to the severe, life-threatening condition called pseudomembranous colitis. The antimicrobials most often implicated are clindamycin, ampicillin, and cephalosporins; however, *C. difficile* intestinal disease can occur following exposure to a wide variety of antimicrobials. Currently, therapy for patients with antibiotic-induced *C. difficile* intestinal disease includes treatment with vancomycin or metronidazole, agents which inhibit the growth of *C. difficile*, but treatment failures and relapse of disease remain a problem. Therefore, more effective agents are required that are not prone to relapse and are efficacious against the more virulent BI/NAP1 epidemic strains.

Methods and Materials

Organisms: *Clostridium difficile* UNT103-1 (VA11 - clinical isolate) and UNT106-1 (VA5 - epidemic BI/NAP1 clinical isolate).
In vitro: MICs were determined in accordance with CLSI guidelines for anaerobic organisms.
Animals: Male Golden Syrian hamsters, 80 – 100 gm.
HCDAD: On day -1, all hamsters were infected by oral gavage with the *C. difficile* culture. Culture was prepared from TSA+SB (5%) plates suspended into pre-reduced TGY (nutrient) broth & anaerobically incubated at 37°C for 24 hours. At 24 hours, cultures were diluted 10-fold into SM (sporulation) broth and anaerobically incubated at 37°C for 48 hours. On the day of infection, cultures were adjusted to ~ 1 x 10^8 CFU/mL in pre-reduced SM broth and 0.5 mL was administered orally to each hamster. At 24 hrs after infection, all animals received a single subcutaneous injection of clindamycin (10 mg/kg).
Treatment: SMT19969 & SMT21829 (10, 20, 50 mg/kg) and vancomycin (20 mg/kg) were administered orally (N = 5 or 10) starting 48 hrs after infection and continued once daily for 5 days. Survival was monitored out to 21 days.
Sampling: Contents of the cecum were collected from any animal that died on study and from all remaining animals on day 21. All cecal contents were assayed for the presence of *Clostridium difficile* Toxins A and B using the Wampole *C. Difficile* Tox A/B II ELISA assay kit in accordance with manufacturers directions.
Pharmacokinetics: SMT19969, 20 mg/kg, qd x 5 days. Plasma and cecum samples were taken at 1, 3 and 5 hrs on days 1 and 5 and assayed for SMT19969 by LCMS methods.

Panel 1. Chemical Structures



SMT19969 and SMT21829 chemical scaffold

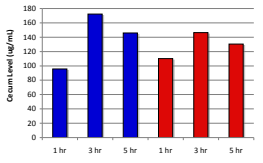
Vancomycin HCl structure

Panel 2. Minimum Inhibitory Concentrations (MICs) of Selected Agents Against *C. difficile* Isolates

	MIC (ug/mL)	
Compound	UNT103-1	UNT106-1
SMT19969	0.25	0.25
SMT21829	0.125	0.25
Vancomycin	0.5	1.0
Metronidazole	≤ 0.5	1.0

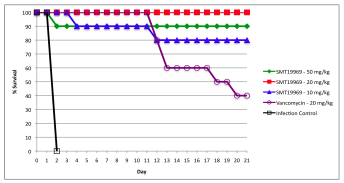
- SMT19969 and SMT21829 were both approx. 2-4 fold more active than either vancomycin or metronidazole against clinical isolates of *C. difficile* including the BI/NAP1 strain (UNT106-1).

Panel 3. Pharmacokinetics of SMT19969 in *C. difficile* Infected Hamsters



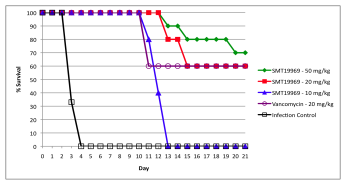
- SMT19969 was administered orally at 20 mg/kg 1xday for 5 days.
- Corresponding plasma levels were < LOQ (25 ng/mL).

Panel 4. Efficacy of SMT19969 against the *C. difficile* Clinical Isolate UNT103-1



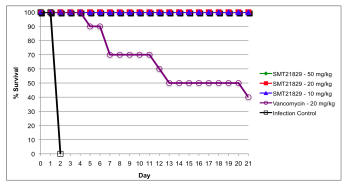
- For SMT19969, 90%, 100% and 80% of the animals survived at the 50, 20 and 10 mg/kg dose groups, respectively.
- Vancomycin exhibited 100% protection during treatment with relapse observed starting on Day 12 and continuing through Day 20 with 40% survival at the end of the study.

Panel 5. Efficacy of SMT19969 against the epidemic BI/NAP1 Clinical Isolate UNT106-1



- All animals administered 20 and 50 mg/kg SMT19969 survived during treatment with a stepwise mortality observed starting on day 12 resulting in 60 – 70% survival at the end of the study.
- Relapse with Vancomycin occurred earlier on day 11 with 60% survival by Day 21.

Panel 6. Efficacy of SMT21829 against the *C. difficile* Clinical Isolate UNT103-1



- All animals administered SMT21829 (50, 20 or 10 mg/kg) survived until the end of the study (Day 21).
- Animals treated with Vancomycin exhibited mortality starting on Day 5 of dosing (10% mortality) and continued in a stepwise manner through Day 21 with 40% survival at the end of the study.

Panel 7. Wampole Tox A/B Elisa Results for SMT19969 against the *C. difficile* Clinical Isolate UNT103-1

		Tox A/B Results Animal #									
Treatment	Dose (mg/kg)	1	2	3	4	5	6	7	8	9	10
SMT19969	50	+	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-
	10	+	+	+	+	+	+	+	+	+	+
Vancomycin	20	+	+	+	+	+	+	+	+	+	+
Control	NA	+	+	+	+	+	+	+	+	+	+

Shaded area indicates animal died on study

- With the exception of two animals administered 10 mg/kg SMT19969[a], all animals that died on study were positive for clostridial Toxins A & B , whereas all survivors were toxin negative.

[a] due to gavage error and cecal sample size

Panel 8. Wampole Tox A/B Elisa Results for SMT19969 against the epidemic BI/NAP1 Clinical Isolate UNT106-1

		Tox A/B Results Animal #				
Treatment	Dose (mg/kg)	1	2	3	4	5
SMT19969	50	-	+	-	-	-
	20	+	-	-	-	+
	10	+	+	+	+	+
Vancomycin	20	+	-	+	+	-
Control	NA	+	+	+	+	+

Shaded area indicates animal died on study

- With the exception of one animal administered 20 mg/kg SMT19969[a], all animals that died on study were positive for clostridial Toxins A & B, whereas all survivors were toxin negative.

[a] due to cecal sample size

Panel 9. Wampole Tox A/B Elisa Results for SMT21829 against the *C. difficile* Clinical Isolate UNT103-1

		Tox A/B Results Animal #									
Treatment	Dose (mg/kg)	1	2	3	4	5	6	7	8	9	10
SMT21829	50	-	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-
Vancomycin	20	+	+	+	+	+	+	+	+	+	+
Control	NA	+	+	+	+	+	+	+	+	+	+

Shaded area indicates animal died on study

- All SMT21829 treated animals were negative for the presence of Toxins A & B.
- Vancomycin and Control animals that died on study were all toxin positive, while all survivors were toxin negative.

Summary and Conclusions

- SMT19969 and SMT21829 were 2 to 4-fold more active *in vitro* against the non-epidemic and BI/NAP1 *C. difficile* isolates than either vancomycin or metronidazole.
- Against the non-epidemic strain infection, SMT21829 administration resulted in complete survival of infected animals while SMT19969 administration resulted in 80 – 100% survival through day 21 over the dose ranges tested. Vancomycin administration resulted in only 40 – 60% survival by day 21.
- In BI/NAP1 infected animals, SMT19969 at 20 and 50 mg/kg exhibited complete protection out to Day 12 with 60% and 70% of the animals surviving from Day 15 through 21, respectively. Vancomycin at 20 mg/kg also demonstrated 60% survival by the end of the study, but relapse initiated earlier (Day 11) as compared to SMT19969 dosed animals.
- Peak cecum levels of SMT19969 reached 172 ug/mL at 3 hrs after a single 20 mg/kg oral dose with no apparent accumulation after 5 days of dosing. All plasma levels were below the LOQ indicating low - no bioavailability.
- SMT19969 and SMT21829 exhibited greater efficacy than vancomycin against both the epidemic BI/NAP1 and non-epidemic isolates evidenced by lower relapse and/or delayed time to relapse.
- Efficacy in the hamster CDAD model, coupled with their antimicrobial profile, makes the SMT compounds excellent candidates for further testing as agents for the treatment of *Clostridium difficile* associated disease.

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