SMT19969: Preclinical Safety and Pharmacokinetics of a Novel Antibiotic for *Clostridium difficile* Infection

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Introduction and Purpose

C. difficile infection (CDI) is now a significant cause of mortality and morbidity in both the primary and wider healthcare setting.[1] Current standard-of-care therapy is complicated by recurrent disease, which occurs in up to 30% of patients following initial infection, with risk of further recurrence and disease severity increasing with each subsequent episode.[2] Therapy options are limited, with Vancocin and Dificid being the only FDA approved antibiotics. Recent Phase III trials have shown Dificid to be non-inferior on initial cure when compared to vancomycin and it is associated with reduced rates of recurrent disease.[3]

SMT19969 is a narrow spectrum antibiotic in clinical development for the treatment of CDI. The objectives of the following GLP studies were to assess the toxicology, pharmacokinetics and distribution of SMT19969 to allow initiation of Phase I clinical trials.

Methods

Studies were carried out by Covance Laboratories under GLP conditions and in accordance with ICH guidelines. **28 Day Repeat Dose Studies:** SMT19969 was administered orally once daily for 28 days to dogs (N=3M+3F) and rats (N=10M+10F) at doses of 1,000mg/kg. Animals were monitored daily for general health and food consumption. Blood samples for toxicokinetics and clinical pathology were taken on days 1 and 28. On completion of dosing, all animals were subject to necropsy with organ weights recorded and tissues examined by histopathology. **QWBA:** Single 50mg/kg dose of [^{14}C]-SMT19969 administered to male albino rats (N=4). Animals sacrificed at 1, 4, 8, and 24 hours post-dose with a terminal bleed for plasma analysis. GI tracts were ligated, removed, and separated into stomach, small intestine, caecum and large intestine and contents flushed with saline for radioanalysis. Abdominal cavities were filled with embedding fluid and sutured. Carcasses and gut samples were snap frozen, sectioned and analysed for radioactivity. **Mass Balance Recovery:** Single 50mg/kg dose of ^{14}C SMT19969 administered to male albino rats (N=3) individually housed in metabolism cages. Urine, faeces and cage wash/debris was collected at defined intervals and analysed for radioactivity. **Irwin:** Effects on general activity, behaviour, autonomic and motor effects were assessed by Irwin's method following IV administration of SMT19969 (0.5, 1.5, 3.0 mg/kg) to male rats (N=6). Observations were performed and body temperatures recorded at 5, 15, 30, 60 and 120 minutes post dosing. Animals were monitored for a further 7 days for gross signs of toxicity. **Cardiovascular and Respiratory Study:** The effects of intravenously administered SMT19969 on cardiovascular and respiratory function was assessed in anaesthetised male dogs. Animals (N=4 per group) were administered ascending doses of SMT19969 (0.5, 1, 2mg/Kg) or the same number of doses of vehicle by slow infusion over 10 minutes with at least 25 minutes between each dose. Animals were instrumented for measurement of blood pressure, heart rate, left ventricular pressure, artery blood flow, RR, QRS, PR, QT and QT_C-intervals and height of the R wave of the ECG complex, peak expiratory and inspiratory flow, tidal volume, minute volume and respiratory rate. **hERG Inhibition:** Whole-cell patch-clamp was used to investigate the effects of SMT19969 on hERG potassium channels stably expressed in HEK 293 cells at physiological temperature. The selective IKr blocker E-4031. was included as positive control. SMT19969 was assayed to the limit of solubility. ***In vitro* Genotoxicity:** Assessed *in vitro* in bacterial Ames and chromosomal aberration assays in the presence and absence of metabolic activation (S-9 fraction) to the limit of solubility. Negative (vehicle) and standard positive controls were included. Bacterial reverse mutation assay used four histidine-requiring *S. typhimurium* strains (TA98, 100, 1535, 1537) one tryptophan-requiring *E. coli* strain (WP2 uvrA). Cytogenetics assays were carried out in duplicate human lymphocyte cultures prepared from the pooled blood of three female donors in two independent experiments. ***In vivo* Genotoxicity:** *In vivo* assessment was carried out by two methods. Induction of DNA damage in the colon of rats following oral dosing was assessed by a Comet assay on colon tissue. Male rats (N=6 per group) received two doses (21 hours apart) by oral gavage of SMT19969 (200, 1000 or 2000 mg/Kg), vehicle or EMS positive control (20mg/Kg). 24 hours post first dose, animals were sacrificed and single cell suspensions from colon tissue assessed by single cell gel electrophoresis for DNA damage. Induction of micronuclei was assessed in the polychromatic erythrocytes (PCE) of the bone marrow of treated rats. Male rats (N=6 per group) received single IV doses of SMT19969 (0.5 or 2.5 mg/Kg), vehicle or CPA positive control (20mg/Kg). Animals were sacrificed at either 24 or 48 hours post dose with bone marrow isolated and analysed for micronuclei. Satellite groups of male rats (N=3) were administered SMT19969 to establish exposure levels.

References

1. Rupnik, M., Wilcox, M. H., and Gerding D. N. Nat. Rev. Microbiol., 2009, **7**, p526-536
2. McFarland, L. V., Elmer, G. W., Surawicz, C. M., Am. J. Gastro., 2002, **97**, p1769–1775.
3. Louie, T. J., Miller, M. A., Mullane, K. M., Weiss, K, Lentnek, A., Golan, Y., Gorbach, S., Sears, P., Shue, Y-K., N. Engl. J. Med. 2011, **364**, p422-431.

Results: 28 Day Repeat Dose Toxicology

Toxicity following repeat administration assessed in rat and dog

- Oral administration of 1,000mg/kg/day for 28 days
- No drug related adverse effects, post dosing observations or clinical signs
- No adverse findings on necropsy and histopathology of tissues
- No adverse findings from any in-life assessments including clinical chemistry, haematology, urinalysis and electrocardiogram
- Minimal systemic exposure with plasma levels below the LOQ (1ng/mL)
- NOAEL for oral dosing is set at 1,000mg/kg in the rat and dog

Results: Safety Pharmacology

Effects on hERG channel assessed by whole cell patch clamp

- No significant reduction in tail current when tested to the limit of solubility

General CNS effects were assessed by the Irwin method in rat

- IV administration of SMT19969 at 0.5, 1.5, 3.0 mg/kg
- No behavioural, physiological, motor, autonomic or general behavioural effects observed and no changes in body temperature recorded

Cardiovascular and respiratory function were assessed in anaesthetised dogs

- Escalating IV administration of SMT19969 at 0.5, 1 and 2 mg/kg
- No statistically significant changes recorded in cardiovascular or respiratory function and no effect on QT interval was observed

Results: *In vitro* Genotoxicity

Genotoxicity was assessed *in vitro* in Ames and chromosomal aberration assays

- No evidence for genotoxicity was seen in either of the *in vitro* systems both in the absence or presence of metabolic activation (S9 fraction)
- No increase in the number of revertants in Ames assays
- No chromosomal aberrations in cultured human peripheral blood lymphocytes.

Results: *In vivo* Genotoxicity

Genotoxicity was assessed *in vivo* by induction of micronucleated polychromatic erythrocytes (MN PCEs) in the bone marrow of rats and by assessment of DNA strand breaks in the colon of rats using a Comet assay.

- No evidence for genotoxicity was seen in either of the *in vivo* system
- No increase in MN PCEs with IV administration at 0.5 or 2.5 mg/kg
- No increase in tail intensity and tail movement on Comet assessment of GI tissue following oral administration of SMT19969 at doses up to 2,000 mg/kg
- Plasma levels following IV administration >2,000 fold higher than from oral dosing with no acute toxicity

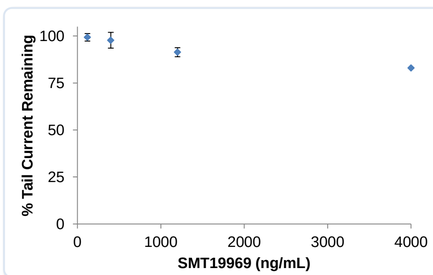

Figure 1: hERG Inhibition Following SMT19969 Treatment (±SEM).

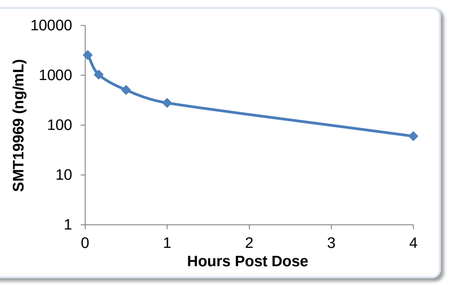

Figure 2: Concentrations of SMT19969 (ng/mL) in Plasma Following IV Administration at 2.5mg/Kg

Results: QWBA and Excretion Mass Balance

Tissue distribution and GI concentrations of ^{14}C-labelled SMT19969 were assessed in rats following a single oral dose of 50 mg/kg

- Drug concentrations >1,000 fold higher than MIC achieved in ligated sections of the GI tract following a single oral dose of SMT19969
- No radioactivity detected in any tissue outside of the GI tract
- >99.5% of radioactivity recovered in faeces
- >99% of radioactivity excreted by 48 hours post dose

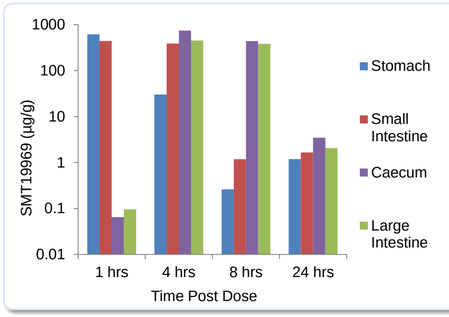

Figure 3: Concentrations of SMT19969 (µg/g) in the Contents of Ligated GI Sections Over Time

Sample	Cumulative Mean % Recovery	
	48 hrs	168 hrs
Urine	0.027	0.027
Faeces	99.6	99.8
Cage wash	0.006	0.012
Carcass	-	1.94
Mean total	99.6	101.8

Table 1: Cumulative Mean % Recovery of SMT19969 at 48 and 168 Hours Post Dose

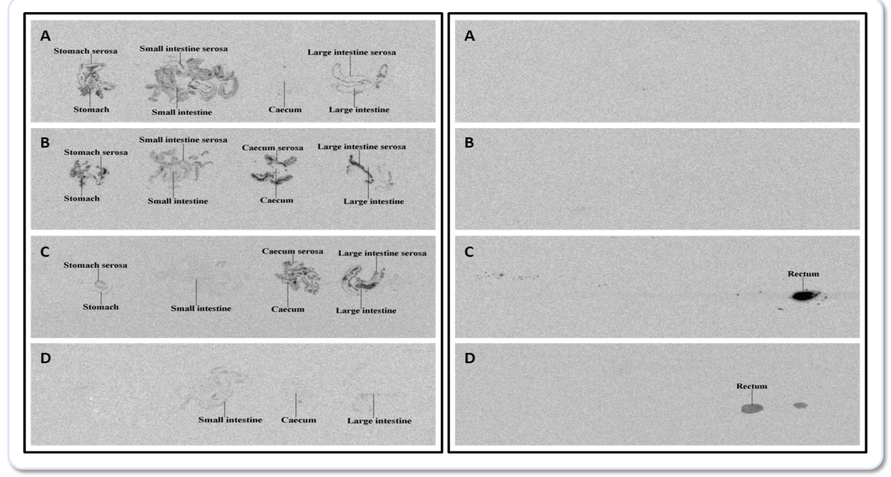

Figure 3: Left Panel – Representative Autoradiograms for Sectioned GI Tissues. Right Panel – Representative Autoradiograms for Carcasses Following Removal of GI Tract. A: 1 hour B: 4 hours C: 8 hours D: 24 hours post dose

Conclusions

- SMT19969 was shown to be well tolerated following 28 days repeat oral dosing at 1,000mg/kg (maximum feasible dose)
- Oral dosing resulted in minimal systemic exposure
- No evidence of genotoxicity in a battery of *in vitro* and *in vivo* tests
- No issues identified from safety pharmacology studies
- These data support the continued clinical development of SMT19969 as a potential therapy for CDI.