F-1512

Time-kill and Post-antibiotic Effect Studies of a Seachaid Pharmaceuticals Investigational Compound (SP2078) Against Wildtype and Multidrug-resistant Pathogens

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ICAAC 2012
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ABSTRACT

Background: SP2078 is a new glycopeptide antimicrobial agent. In this study, time-kill and post-antibiotic effect (PAE) experiments were performed to evaluate the *in vitro* activity of SP2078 tested against selected strains.

Methods: One strain each of wildtype methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant (MRSA) USA100 and USA300, vancomycin-intermediate *S. aureus* (VISA), wildtype *S. epidermidis*, wildtype *E. faecalis*, wildtype *E. faecium*, VanA-*E. faecium*, VanB-*E. faecalis* and wildtype *S. pyogenes* strains were included. SP2078 and vancomycin susceptibility testing and time-kill assays were performed according to CLSI guidelines. SP2078 assays used polysorbate-80 (0.002%). SP2078 was tested at fixed concentrations of 0.5, 1, 2, 4, 8 and 16 μg/mL, while vancomycin was assayed at concentrations resembling free peak (standard doses; 4, 8 and 16 μg/mL). Cell counts were determined by serial dilution plating.

Results: SP2078 displayed bactericidal activity (≥3 log kill relative to starting inoculum) within the testing period (24h) for MSSA (at ≥2 μg/mL) and MRSA (≥4 μg/mL) strains. Bacteriostatic patterns were noted for SP2078 at lower concentrations tested. Vancomycin showed cidality against MSSA (at 16 μg/mL) and MRSA (4-16 μg/mL) within 24h. A killing effect was obtained for SP2078 at 8 and 16 μg/mL against a VISA strain within 24h, while static results were noted for this drug at ≤4 μg/mL. Vancomycin was inactive (4 μg/mL) or static (8 and 16 μg/mL) against VISA. All SP2078 concentrations tested against *S. epidermidis* and *S. pyogenes* displayed cidal profiles, whereas vancomycin exhibited cidal and static time-kill curves against these strains, respectively. All SP2078 concentrations tested showed static results against enterococci, except against a VanA-*E. faecium* strain, where similar results were obtained at higher concentrations (4-16 μg/mL). Overall, vancomycin was inactive against enterococci, with static activity against wildtype *E. faecalis* only. SP2078 had prolonged PAE when compared to vancomycin, regardless of concentration or strain tested.

Conclusions: These results suggest that SP2078 possesses a concentration-dependent killing of MSSA (at ≥2 μg/mL), MRSA (≥4 μg/mL) and VISA (≥8 μg/mL). In contrast, killing activity was noted for all SP2078 concentration tested against *S. epidermidis* and *S. pyogenes*. A static effect was noted for SP2078 when tested against enterococci.

INTRODUCTION

Multidrug-resistant (MDR) isolates of *Staphylococcus aureus* and enterococci, mainly *Enterococcus faecium*, have increasingly been identified in both hospital and community settings. Infections caused by these pathogens pose a clinical threat for antimicrobial therapy, create a significant economic burden, and are associated with increased morbidity and mortality. Methicillin-resistant *S. aureus* (MRSA) now represents more than 50% of *S. aureus* isolates responsible for infections in USA intensive care units. In addition, community-acquired MRSA strains belonging to the USA300 clone currently represent the vast majority of *S. aureus* responsible for skin and skin-structure infections outside of USA hospitals. However, increasing incidences of nosocomial infections caused by USA300 have been reported. These CA-MRSA strains have also been responsible for severe cases of pneumonia.

Vancomycin has long been the gold standard agent for the empirical management of serious Gram-positive infections, including those caused by MRSA in hospitalized patients. However, it has well-recognized limitations, including slow cidality and tolerance, uncertainty regarding the efficacy against heterogeneous vancomycin-intermediate *S. aureus* (hVISA) strains, and variations between and within patients with regards to tissue distribution. Therefore, the increased prevalence of MDR Gram-positive isolates and severe clinical conditions of patients infected by these pathogens underscores the need to identify and develop novel and effective antimicrobial agents. A novel glycopeptide agent (SP2078; see poster F-1510 for additional information) is in preclinical development for the treatment of serious Gram-positive infections. In this study, time-kill and post-antibiotic effect (PAE) experiments were performed to evaluate the SP2078 *in vitro* activity against selected strains.

MATERIALS AND METHODS

Bacterial Isolates. One strain each of wildtype methicillin-susceptible *S. aureus* (MSSA; American Type Culture Collection [ATCC] 29213), MRSA USA100 (NRS382) and USA300 (NRS384), VISA (NRS17), wildtype *Staphylococcus epidermidis* (ATCC 12228), wildtype *Enterococcus faecalis* (ATCC 29212), wildtype *E. faecium* (ATCC 35667), VanA-*E. faecium* (ATCC 51559), VanB-*E. faecalis* (ATCC 51299) and wildtype *Streptococcus pyogenes* (ATCC 19615) strains were included in the time-kill curve experiments. PAE assays were performed using selected bacteria, as follows: one strain each of MSSA (ATCC 29213), MRSA USA100 (NRS382) and USA300 (NRS384, *S. epidermidis* (ATCC 12228) and *S. pyogenes* (ATCC 19615).

Antimicrobial susceptibility testing (Table 1). Isolates were tested for susceptibility by broth microdilution methods following the Clinical and Laboratory Standards Institute (CLSI; M07-A9, 2012) document. Solvent, diluents and dilution procedures utilized for SP2078 investigational compound followed the CLSI recommendations for water-insoluble agents (Table 7B; M100-S22, 2012). Susceptibility testing was performed in cation-adjusted Mueller-Hinton broth (CA-MHB) using customized frozen-form 96-well panels. CA-MHB was supplemented with the surfactant polysorbate-80 (P-80; final well concentration, 0.002%) and isolates were tested in triplicate. Quality assurance was performed by concurrent testing of CLSI-recommended (M100-S22, 2012) quality control (QC) strains: *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213. Interpretations of MIC results obtained for vancomycin tested against QC strains were in accordance with the MIC ranges published in CLSI (M100-S22).

Time-kill curve experiments. Kill curve assays were performed for SP2078 and vancomycin against the organisms described above according to established guidelines (CLSI; M26-A). SP2078 was tested at fixed concentrations of 0.5, 1, 2, 4, 8 and 16 μg/mL, while vancomycin was assayed at 4, 8 and 16 μg/mL. Vancomycin concentrations were chosen to approximate doubling dilutions corresponding to free peak (~16 μg/mL; fC_{max}; defined as the predicted peak level of free [unbound] drug) and free trough levels (~4 μg/mL) in plasma following standard dosages (Vancomycin Package Insert). P-80 was added at final concentration of 0.002% in all *in vitro* assays to minimize drug loss to any labware (plastic/glass) surfaces. Colony counts were performed at T_0, T_2, T_4, T_8 and T_{24}. A control growth was included in each assay. Bactericidal activity was defined as a ≥3 $\log_{10}$ decrease in the bacterial viable cell counts relative to starting inoculum.

PAE experiments. PAE was determined for SP2078 (2, 4, 8 and 16 μg/mL) and vancomycin (4, 8 and 16 μg/mL) using concentrations similar to those utilized during the time-kill experiments. Glass tubes containing 5 mL of Mueller Hinton broth, P-80 (0.002%) and the antimicrobial concentrations described above were inoculated with viable bacterial cells to reach a final concentration of approximately 5 x 10^6 CFU/mL. Inoculated glass tubes were incubated at 35°C under shaking for an antimicrobial exposure period of 1 hour. After this period, exposed bacterial cells were centrifuged and the supernatant containing drug removed. Fresh, pre-warmed broth media (5 mL) was added and tubes incubated at 35°C under shaking. Colony counts were performed at T_0 (pre-antimicrobial exposure), T_1 (post-antimicrobial exposure) and after removal of the antimicrobial (centrifugation method), and every subsequent hour until turbidity was observed. A control test tube, where bacterial cells were not exposed to antimicrobial agents was included.

RESULTS

- SP2078 decreased the viable cell counts of *S. aureus* ATCC 29213 by at least 3 $\log_{10}$ relative to starting inoculum within 24 h when tested at 2 and 4 μg/mL, while a 3 $\log_{10}$ reduction was observed within 12-16 h at SP2078 concentration of 8 μg/mL (**Table 2**). Vancomycin showed static activity when tested against ATCC 29213 within 24 h at concentrations of 4 and 8 μg/mL, respectively, but bactericidal activity was observed at 16 μg/mL (24 h).
- When tested against USA100 and USA300 strains, SP2078 yielded a 3 $\log_{10}$ decrease in the viable cell counts at concentrations of 4 (24 h), 8 (12-16 h) and 16 μg/mL (4-8 h; **Table 2**). Lower SP2078 concentrations demonstrated bacteriostatic results against USA100 and USA300 strains within 24 h. Vancomycin provided a 3 $\log_{10}$ decrease within 24 h at all tested concentrations.
- The SP2078 compound was bactericidal when drug concentrations of 16 (12-16 h) and 8 μg/mL (24 h) were utilized against a VISA strain (**Table 2**). SP2078 tested at concentrations of ≤4 μg/mL showed bacteriostatic activity. Vancomycin was inactive (at 4 μg/mL) or bacteriostatic (8 and 16 μg/mL) when tested against this VISA (**Table 2**).
- All SP2078 concentrations tested against the *S. epidermidis* ATCC 12228 strain exhibited bactericidal activity within 24 h, as did vancomycin (**Table 2**).
- When SP2078 was tested against the *S. pyogenes* ATCC 19615 strain, all concentrations decreased the viable cell counts ≥3 $\log_{10}$ within 4 to 24 h (**Table 2**), whereas vancomycin demonstrated bacteriostatic activity (1.9-2.2 $\log_{10}$ decrease).
- A significant reduction in the viable cell counts (≥3 $\log_{10}$) was not observed when the investigational compound SP2078 was tested against a wildtype strain of *E. faecium* (ATCC 35667; **Table 2**). Similar results were noted for vancomycin, except when tested at a concentration resembling the fC_{max} (16 μg/mL), where cidality was observed at 24 h.
- The SP2078 concentrations of 4, 8 and 16 μg/mL produced a bacteriostatic effect against a vancomycin-resistant VanA-type *E. faecium* strain (ATCC 51559; **Table 2**). Lower SP2078 concentrations tested did not inhibit the growth of this strain.
- SP2078 and vancomycin exhibited bacteriostatic activity when tested against a vancomycin-susceptible *E. faecalis* strain (ATCC 29212), regardless of the antimicrobial concentration tested (**Table 2**).
- When tested against a vancomycin-resistant strain of *E. faecalis* exhibiting a VanB phenotype, all SP2078 concentrations tested inhibited the bacterial growth (0.8-1.3 $\log_{10}$ reduction; **Table 2**). Vancomycin was inactive against this strain (*E. faecalis* ATCC 51299).

Table 1. Modal minimum inhibitory concentration values for SP2078 and vancomycin tested in triplicate against selected Gram-positive strains.

Organism		MIC (μg/mL)	
Isolate number	Phenotype	SP2078	Vancomycin
S. aureus			
ATCC 29213	Wildtype	0.06	1
NRS382 (USA100)	Methicillin-resistant	0.12	2
NRS384 (USA300)	Methicillin-resistant	0.06	1
NRS17	Vancomycin-intermediate	0.12	4
S. epidermidis			
ATCC 12228	Wildtype	0.06	1
S. pyogenes			
ATCC 19615	Wildtype	0.06	0.5
E. faecium			
ATCC 35667	Wildtype	0.03	0.5
ATCC 51559	Vancomycin-resistant (VanA)	4	>64
E. faecalis			
ATCC 29212	Wildtype	0.06	2
ATCC 51299	Vancomycin-resistant (VanB)	0.12	64

- SP2078 demonstrated PAE results from 2.0 to 3.6 h when tested at doubling dilution concentrations ranging from 2 to 16 μg/mL against selected *S. aureus* strains (**Table 3**). Vancomycin provided an overall PAE range of 0.5-1.2 h against these *S. aureus* strains.
- SP2078 tested at concentrations of 2, 4 and 8 μg/mL inhibited the growth (<1 $\log_{10}$ in viable cell counts) of a *S. epidermidis* ATCC 12228 strain for ~4 h, while a SP2078 concentration of 16 μg/mL inhibited growth for ~8 h (**Table 2**). Vancomycin demonstrated a PAE between 1.7 and 2.3 h when tested against this *S. epidermidis* strain.
- A PAE varying from 1.6 to 2.9 h was observed for a wildtype strain of *S. pyogenes* (ATCC 19615) when exposed for 1 hour to this investigational compound SP2078 (**Table 3**). Vancomycin exhibited a PAE of three to six times shorter (0.5 h) against this strain.

Table 3. Post-antibiotic effect results (in hours) for SP2078 and vancomycin tested against selected Gram-positive strains.

Organism		PAE at exposure concentration (μg/mL) of:						
		SP2078				Vancomycin		
Isolate number	Phenotype	2	4	8	16	2	4	8
S. aureus								
ATCC 29213	Wildtype	2.8	3.5	3.2	3.2	1.2	1.2	1.2
NRS382 (USA100)	Methicillin-resistant	3.3	3.0	3.0	3.6	0.5	0.6	0.6
NRS384 (USA300)	Methicillin-resistant	2.2	2.0	2.3	2.5	0.6	0.5	0.5
S. epidermidis								
ATCC 12228	Wildtype	4.0	4.0	4.6	8.0	1.7	2.3	2.0
S. pyogenes								
ATCC 19615	Wildtype	1.6	2.9	2.4	2.9	0.5	0.5	0.5

Table 2. Time-kill curve experiments for SP2078 and comparator agent, vancomycin, tested against selected Gram-positive strains.

Organism		Concentration (μg/mL) of:								
		SP2078[a]						Vancomycin[a]		
Isolate number	Phenotype	0.5	1	2	4	8	16	4	8	16
S. aureus										
ATCC 29213	Wildtype	-1.6 $\log_{10}$	-1.1 $\log_{10}$	**24h**	**24h**	**12-16h**	NT	-0.6 $\log_{10}$	-2.5 $\log_{10}$	**24h**
NRS382 (USA100)	Methicillin-resistant	-1.6 $\log_{10}$	-1.8 $\log_{10}$	-2.2 $\log_{10}$	**24h**	**12-16h**	**4-8h**	**20-24h**	**20-24h**	**20-24h**
NRS384 (USA300)	Methicillin-resistant	-1.4 $\log_{10}$	-1.4 $\log_{10}$	-0.8 $\log_{10}$	**24h**	**12-16h**	**4-8h**	**20-24h**	**20-24h**	**20-24h**
NRS17	Vancomycin-intermediate	-2.3 $\log_{10}$	-2 $\log_{10}$	-2.0 $\log_{10}$	-2.4 $\log_{10}$	**24h**	**12-16h**	*+3.2 $\log_{10}$*	-1.5 $\log_{10}$	-1.4 $\log_{10}$
S. epidermidis										
ATCC 12228	Wildtype	**12-16h**	**8-12h**	**8-12h**	**4-8h**	**4-8h**	**2-4h**	**12-16h**	**8-12h**	**8-16h**
S. pyogenes										
ATCC 19615	Wildtype	**24h**	**16-20h**	**12-16h**	**12-16h**	**8-12h**	**4-8h**	-1.9 $\log_{10}$	-2.0 $\log_{10}$	-2.2 $\log_{10}$
E. faecium										
ATCC 35667	Wildtype	-0.2 $\log_{10}$	-0.2 $\log_{10}$	-0.2 $\log_{10}$	-0.2 $\log_{10}$	-0.4 $\log_{10}$	-2.0 $\log_{10}$	0.0 $\log_{10}$	0.0 $\log_{10}$	**24h**
ATCC 51559	Vancomycin-resistant (VanA)	*+2.2 $\log_{10}$*	*+2.1 $\log_{10}$*	*+1.6 $\log_{10}$*	-0.3 $\log_{10}$	-0.8 $\log_{10}$	-2.0 $\log_{10}$	*+2.4 $\log_{10}$*	*+2.5 $\log_{10}$*	*+2.5 $\log_{10}$*
E. faecalis										
ATCC 29212	Wildtype	-0.7 $\log_{10}$	-0.5 $\log_{10}$	-0.7 $\log_{10}$	-1.1 $\log_{10}$	-0.9 $\log_{10}$	-1.0 $\log_{10}$	-1.8 $\log_{10}$	-0.9 $\log_{10}$	-0.7 $\log_{10}$
ATCC 51299	Vancomycin-resistant (VanB)	-0.8 $\log_{10}$	-1.2 $\log_{10}$	-1.3 $\log_{10}$	-1.3 $\log_{10}$	-0.8 $\log_{10}$	-0.9 $\log_{10}$	*+2.5 $\log_{10}$*	*+2.5 $\log_{10}$*	*+2.5 $\log_{10}$*

a. Bolded results represent time (approximate hours to cidality) when bactericidal activity (≥3 $\log_{10}$ cfu/mL decrease in cell density relative to starting inoculum) was observed; Underlined values represent static activity (0 to <3 $\log_{10}$ decrease in cfu/mL at 24 hours relative to starting inoculum); Results in italic represent those experiments where inhibition of growth was not observed (>0.1 $\log_{10}$ increase in cfu/mL at 24 hours relative to starting inoculum). NT, reads concentration not tested.

CONCLUSIONS

- Overall, SP2078 demonstrated bacteriostatic activity when tested at lower concentration (0.5-2 μg/mL) against *S. aureus*. However, bactericidal activities were observed when SP2078 was tested at concentrations of ≥4 μg/mL against *S. aureus*, including MDR strains.
- The time (hours) required for SP2078 to exhibit a killing effect against *S. aureus* decreased as drug concentration increased, a feature also observed against selected *S. epidermidis* and *S. pyogenes* strains included in this study. These results indicate that SP2078 may possess a concentration-dependent cidal activity.
- SP2078 demonstrated bacteriostatic activity when tested against vancomycin-susceptible and -resistant (VanA and VanB phenotypes) enterococcal strains. In addition, SP2078 had a prolonged PAE when compared to vancomycin, regardless of concentration or strain tested.

ACKNOWLEDGMENTS

The study experiments were conducted at JMI Laboratories (North Liberty, Iowa, USA) and supported by an Educational/Research Grant from Seachaid Pharmaceuticals, Inc. (Durham, North Carolina, USA). JMI Laboratories received compensation fees for services in relation to preparing the abstract/poster, which was funded by Seachaid Pharmaceuticals.

The authors thank the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) Program supported under NIAID/NIH Contract No. HHSN272200700055C for providing the MRSA and VISA strains utilized in this study.

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