Evaluation of PPI-0903 (TAK-599), a Novel Cephalosporin: Bactericidal Activity, Effects of Modifying In Vitro Testing Parameters and Optimization of Disk Diffusion Tests

F-334

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ABSTRACT

Background: PPI-0903M (the bioactive metabolite of PPI-0903) is a novel *N*-phosphono cephalosporin with potent in vitro activity against oxacillin-resistant *S. aureus* and many other Gram-positive organisms, while retaining activity against many Gram-negative bacilli. We evaluated bactericidal action of PPI-0903M and in vitro testing parameters for this compound.

Methods: 110 isolates had PPI-0903M MIC and MBC determined by NCCLS reference methods. Kill-curve experiments were performed on 17 strains at 1X, 2X, 4X and 8X MIC. Time points include T_0, T_4, T_8 and T_{24}. Broth microdilution results for 9 strains (in triplicate) were compared to the standard method after modifying broth pH (5.0, 6.0, 7.2 and 8.0), incubation environments (air, 5% CO_2, and anaerobic), inoculum size (10^3, 10^5, 10^7 CFU/ml), Ca^{++} content (25 and 50 mg/L) and human serum (HS) concentration in the broth (0, 5, 10 and 50%). Optimal disk content was assessed by testing 7 ATCC strains with 5 PPI-0903M disks (5, 10, 30, 50 and 100-µg).

Results: PPI-0903M generally demonstrated bactericidal activity at or one $\log_2$ dilution above the MIC for 86.4% of tested organisms. 90% of strains had MBC/MIC ≤ 4. An eagle-effect was noted with some isolates. Kill-curve studies showed 99.9% killing at concentration ≥ 4X MIC within 8 to 24 hours against staphylococci and *Enterobacteriaceae* tested. MIC results were markedly lower at pH 5.0 than at baseline due to suboptimal growth. PPI-0903M MIC results were rarely influenced by other test or medium changes evaluated. No significant variation (>1 $\log_2$ dilution) was detected using 3 HS concentrations. The 10-µg disk content would be recommended for potential correlate MIC breakpoints (BKP) of 1 - 4 µg/ml, while the 30-µg should be used for BKPs over the 1 - 16 µg/ml interval.

Conclusions: PPI-0903M was bactericidal against all tested species, with MBC values near the measured MIC result. Overall, the PPI-0903M MIC results were very stable across numerous susceptibility test condition changes and HS proteins did not adversely influence activity.

INTRODUCTION

Gram-positive bacterial pathogens have shown a remarkable ability to develop resistance to antimicrobial agents. Oxacillin/methicillin- and glycopeptide-resistant staphylococci, glycopeptide-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae* and viridans group streptococci have forced clinicians to seek alternative treatments for patients with serious Gram-positive infections.

Hospital infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) represent an important worldwide problem, and more recently this pathogen has been increasingly isolated from community-acquired infections.

PPI-0903 (formerly TAK-599) is a novel *N*-phosphono type prodrug cephalosporin (Figure 1). PPI-0903M is the active metabolite of PPI-0903. Preliminary in vitro studies have indicated that this compound has a high affinity for PBP2' or PBP2A and shows potent in vitro activity against MRSA and many other Gram-positive organisms, while retaining activity against many Gram-negative bacilli. We evaluated the bactericidal action of PPI-0903M and in vitro testing parameters for this compound.

MATERIALS AND METHODS

A total of 110 strains were tested to determine their MIC and MBC values. MIC values were determined by NCCLS reference broth microdilution methods. PPI-0903M reagent grade compound was provided by Peninsula Pharmaceutical Inc. (Alameda, CA). Concurrent QC was performed by testing the following control strains: *S. pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

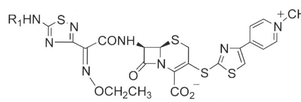
The MBC was assessed by plating the broth from the MIC well and from those wells three $\log_2$ dilutions above the MIC for each organism onto appropriate growth media. Quantitative colony counts were performed on the starting inoculum. The MBC was defined as the lowest concentration of antimicrobial agent that kills ≥ 99.9% of the starting test inoculum.

Seventeen strains (six *S. aureus,* two *S. epidermidis*, four *S. pneumoniae,* and one each of *Haemophilus influenzae*, *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Serratia marcescens*) were tested by the kill-curve methodology to evaluate the bactericidal activity of PPI-0903M. Bacterial kill curves were performed in Mueller-Hinton broth (MHB) for all isolates except *S. pneumoniae* (MHB supplemented with 2-5% lysed horse blood) and *H. influenzae* (Haemophilus Test Medium broth). PPI-0903M activity was tested at timed intervals of T_0, T_4, T_8 and T_{24} hours at 1X, 2X, 4X, and 8X the MIC.

Broth microdilution results for 9 strains (in triplicate) were compared to the standard method after modifying broth pH (5.0, 6.0, 7.2 and 8.0), incubation environments (ambient air, 5% CO_2, and anaerobic), inoculum size (10^3, 10^5 and 10^7 CFU/ml), Ca^{++} content (trace, 25 and 50 mg/L) and human serum concentration in the broth (0, 5, 10 and 50%).

Optimal disk content was assessed by testing seven reference strains with five PPI-0903M disk concentrations (5, 10, 30, 50 and 100 µg). The strains tested in this experiment were: *S. aureus* ATCC 25923 and 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *E. faecium* 89-736D. The disk diffusion method was performed according to NCCLS M2-A8 methods. Ceftriaxone and cefepime were tested concurrently as control agents.

Figure 1. Chemical structure of PPI-0903 (formerly TAK-599) and PPI-0903M (T-91825).



1 (TAK-599); R_1=PO(OH)$_2$ (Acetic acid solvate)
2 (T-91825); R_1=H

Table 1. Listing of MBC results compared to the MIC for 110 organisms tested against PPI-0903M.

Organism group (no. tested)		Occurrences at MBC/MIC ratio of: 1	2	4	>4
S. aureus	oxacillin-resistant (10)	6	3	0	1
	oxacillin-susceptible (10)	9	1	0	0
	hVISA (10)	7	2	0	1
Coagulase-negative staphylococci	oxacillin-resistant (10)	8	2	0	0
	oxacillin-susceptible (10)	8	2	0	0
S. pneumoniae	penicillin-susceptible (10)	10	0	0	0
	penicillin-resistant (10)	4	0	0	6
viridans group streptococci	penicillin-susceptible (5)	5	0	0	0
	penicillin-resistant (5)	3	2	0	0
Enterobacteriaceae (20)		12	3	3[a]	2[b]
Quality control strains (10)		6	2	1	1[c]
Total (110)		78	17	4	11
% Cumulative		70.9	86.3	90	100

a. Includes: *E. coli* (one strain) and *S. marcescens* (two strains).
b. Includes: *E. coli* (one strain) and *S. marcescens* (one strain).
c. One strain, *E. faecalis* ATCC 29212.

Table 2. Effects of varying reference NCCLS broth microdilution test conditions on the MIC of PPI-0903M using 15 selected organisms.

Organism		Reference MIC[a]	PPI-0903M MIC (µg/ml) Inoculum (CFU/ml) 5 x 10^3	5 x 10^7	Calcium conc. Trace	50 mg/L	Incubation Anaerobic	5% CO^2	Supplements Lysed HB	HTM	Medium pH 5.0	6.0	8.0
E. coli ATCC 25922		0.06	0.06	0.25	0.03	0.06	0.06	0.06	0.06	0.06	0.12	0.06	0.06
E. cloacae 32-43A		0.5	0.25	>32[b]	0.25	0.5	0.5	0.5	0.25	0.25	0.12	0.5	0.25
P. aeruginosa ATCC 27853		8	4	>32	1	16	4	4	32	8	0.5	2	32
A. baumannii 25-755A		2	2	4	1	2	2	2	2	1	4	2	2
S. aureus	ATCC 25923	0.12	0.12	0.12	0.06	0.06	0.06	0.12	0.12	0.06	≤0.016	0.12	0.12
	ATCC 29213	0.25	0.12	0.25	0.12	0.12	0.25	0.25	0.12	0.12	0.03	0.12	0.25
	VISA 12	1	1	2	1	1	1	1	2	1	0.03	0.5	2
	30-100A	0.5	0.5	1	0.25	0.5	0.5	0.5	0.5	0.5	0.03	0.5	0.5
CoNS	51-81A	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.03	0.25	0.5
	57-353A	0.06	0.06	0.12	0.03	0.06	0.06	0.06	0.06	0.06	≤0.016	0.06	0.06
E. faecalis	ATCC 29212	1	1	2	0.25	0.5	1	1	0.5	0.25	0.06	0.25	1
	33-16A	8	4	8	0.5	4	4	4	4	0.5	0.12	0.5	8
E. faecium 78-464A		4	4	4	0.5	4	4	4	2	1	0.12	1	4
S. pneumoniae ATCC 49619		0.016	0.016	0.016	NT[c]	0.016	0.016	[a]	[a]	0.008	NG[c]	0.016	0.016
viridans gr. strept 35-329A		0.016	0.016	0.016	NT	0.016	0.016	[a]	[a]	0.008	NG	0.008	0.016

a. MIC determined under NCCLS method conditions, usually Mueller-Hinton broth, pH 7.2 - 7.4, Ca^{++} at 25 mg/L, inoculum of 5 X 10^5 CFU/ml incubated in ambient air. Strains requiring 2 - 5% lysed horse blood (*S. pneumoniae* and viridans group streptococci) have been incubated in that medium.
b. Underlined results vary by > four-fold from reference MIC value.
c. NT = not tested and NG = no growth.

Figure 2. In vitro killing kinetic of PPI-0903M versus selected Gram-positive and Gram-negative bacteria.

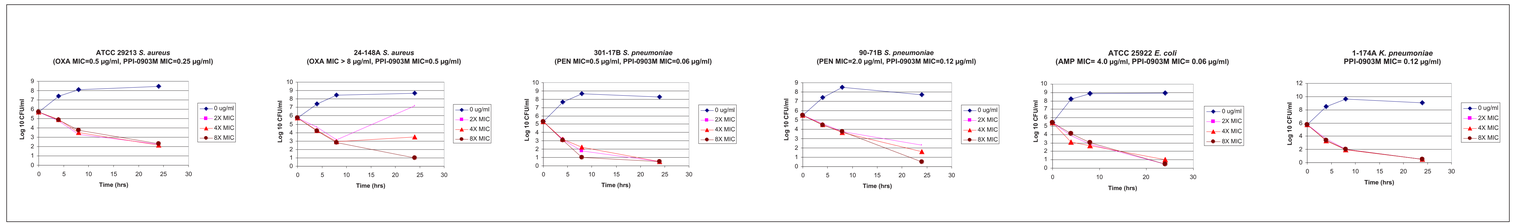
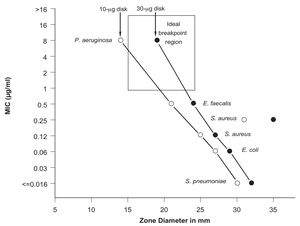


Table 3. Effects of increasing concentrations of human serum on the MIC values of PPI-0903M.

Organism	PPI-0903M MIC (µg/ml) in: Broth	+5% serum	+10% serum	+50% serum
S. pneumoniae ATCC 49619	≤0.016	≤0.016	≤0.016	≤0.016
S. pneumoniae 301-21B	0.12	0.12	0.12	0.12
S. aureus ATCC 29213	0.12	0.12	0.12	0.25
S. aureus 81-4339C	2	1	1	1
S. aureus 33-23A	0.5	0.5	0.5	1
S. aureus 51-76A	0.5	0.5	0.5	1
E. coli ATCC 25922	0.06	0.06	0.06	0.03
K. pneumoniae 1-174A	0.06	0.06	0.03	0.03
P. aeruginosa ATCC 27853	16	32	16	32

Figure 3. Correlation of PPI-0903M zone diameters vs. MICs of QC strains.



RESULTS

- PPI-0903M generally exhibited bactericidal activity at or one $\log_2$ dilution above the MIC for 86.4% of tested organisms. Ninety percent of strains had an MBC/MIC ratio of ≤ 4 (preferred ratios, Table 1).
- By excluding the enterococcal QC and *S. marcescens* results, the bactericidal proportion (ratio at ≤ 4) for PPI-0903M was 92.3%. Therefore, PPI-0903M was considered bactericidal against drug-resistant Gram-positive cocci and most *Enterobacteriaceae* (Table 1).
- Kill-curve kinetic studies confirmed the findings observed using NCCLS MBC determinations for PPI-0903M (Figure 2).
  - Generally bactericidal (≥ three $\log_{10}$ CFU/ml reductions) action was noted for PPI-0903M against staphylococci, although static effects was observed at lower multiples of the reference MIC (2X and 4X) against some isolates.
  - Static and bactericidal action would be expected for *S. pneumoniae*, with greater effect on strains having the lowest PPI-0903M or penicillin MIC results.
  - PPI-0903M was bactericidal versus all *Enterobacteriaceae* (including the *S. marcescens* strain) and *H. influenzae* tested.
- Table 2 summarizes the results from 15 organisms tested against PPI-0903M using 11 variations of the NCCLS method. Only 16 (7.9%) MICs among 165 results varied by > four-fold when compared to the baseline PPI-0903M MIC.
- One test condition was responsible for 9 of 18 significant variations, medium pH at 5.0; results that were markedly lower than baseline MICs due to suboptimal growth (Table 2).
- No significant variation (> ± one $\log_2$ dilution) was detected using MHB supplemented with three human serum concentrations (5, 10 and 50%) and nine organisms representing the streptococci, *Enterobacteriaceae*, non-fermentative Gram-negative bacilli and the staphylococci (Table 3).
- Figure 3 shows the near linear increase in zones of inhibition as one increases the PPI-0903M disk concentrations. Susceptible Gram-positive QC strains all had zone diameters of > 20 mm for 10- to 100-µg disk concentrations, and a correlate MIC of ≤ 0.5 µg/ml. Maximum zone diameter differences (approximately 10 mm) between possible PPI-0903M-resistant strains (*E. faecium*) and susceptible species was achieved with PPI-0903M disk concentrations of 10- or 30-µg.

CONCLUSIONS

- PPI-0903M, the active metabolite of PPI-0903, is a bactericidal cephalosporin against all tested species, with MBC values near the measured MIC.
- Overall, the PPI-0903M MIC results were very stable across numerous susceptibility test technical changes.
- PPI-0903M activity was not adversely influenced by human serum proteins.
- Based on the results from this pilot experiment, the 10-µg disk content would be recommended for potential correlate MIC breakpoints of 1 - 4 µg/ml.
- Further evaluations should be performed to establish the clinical role of this promising cephalosporin.

REFERENCES

Iizawa Y, Nagai J, Ishikawa T, Hashiguchi S, Miyake A, Nakao M, Okonogi K. (2002). TAK-599, a novel N-phosphone type prodrug of anti-MRSA cephalosporin T-91825: In vitro and in vivo antibacterial activity. In: *42nd Interscience Conference on Antimicrobial Agents and Chemotherapy*; San Diego, CA, Sept. 27-30.

Isenberg HD. (1992). Antimicrobial susceptibility testing. In: *Clinical Microbiology Procedures Handbook*. American Society for Microbiology, Washington, DC.

Ishikawa T, Matsunaga N, Tawada H, Kuroda N, Nakayama Y, Ishibashi Y, Tomimoto M, Ikeda Y, Tagawa Y, Iizawa Y, Okonogi K, Hashiguchi S, Miyake A. (2003). TAK-599, a novel N-phosphono type prodrug of anti-MRSA cephalosporin T-91825: Synthesis, physicochemical and pharmacological properties. *Bioorganic and Medicinal Chemistry* 11:2427-2437.

National Committee for Clinical Laboratory Standards. (2003a). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - sixth edition. Approved document M7-A6*. Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2003b). *Performance standards for antimicrobial disk susceptibility tests; Approved standard, 8th ed. Document M2-A8*. Wayne, PA:NCCLS.