

Antimicrobial Activity of Sulopenem in the Urine of Healthy Volunteers

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ABSTRACT

Background: Sulopenem-etzadroxil is a novel oral thiopenem antibiotic being developed for treatment of uncomplicated urinary tract infections (uUTI). It has *in vitro* activity against Enterobacteriaceae, including organisms with extended spectrum beta-lactamase activity. During a phase 1 clinical study in healthy volunteers, urine was collected and tested for activity against key pathogens.

Materials/methods: Patients received 500 mg of oral sulopenem-etzadroxil with or without 500 mg of probenecid, in the fed or fasted state. Sulopenem concentrations in plasma and urine samples were measured. Urine samples from each patient collected in the first 2 hours after dosing were thawed and double-diluted up to 1024-fold in pooled human urine in 96-well plates. Wells were inoculated with exponentially growing bacteria. Urine bactericidal titres and MICs were determined per CLSI guidelines (M21-A & M07-A10).

Results: Mean (median; range) sulopenem urine concentrations were 119.3 µg/mL (84.4; 21.8 -420.0) and 144.9 µg/mL (87.3; 28.8 - 609.0) for the fasted and fed patients, respectively. All undiluted urine samples were bactericidal for all organisms. The table below describes the number of dilutions that still retained bactericidal activity (≥3 log kill).

Organism [Sulopenem MIC]	Maximum fold-dilution of urine retaining bactericidal activity	
	Sulopenem-etzadroxil fasted (n=32)	Sulopenem-etzadroxil fed (n=31)
<i>E. coli</i> ATCC 25922 [0.015 µg/mL]	32	64
<i>E. coli</i> ATCC 35218 [0.015 µg/mL]	128	256
<i>E. coli</i> 1373629 [0.03 µg/mL]	256	256
<i>E. coli</i> 1381423 [0.12 µg/mL]	16	32
<i>K. pneumoniae</i> 1373657* [OXA-48:16 µg/mL]	2	4

n = number of urines tested; *Imipenem and meropenem MICs = 4 µg/mL

Conclusions: Bactericidal activity against all organisms, including the OXA-48-producing organism, was evident in all urine samples tested indicating potent antimicrobial activity of sulopenem in the urine. Sulopenem provided to patients in the fed state was, in most cases, bactericidal at one additional tube dilution relative to those dosed fasted. These data support further study on sulopenem for the treatment of uUTI.

INTRODUCTION

- Sulopenem is a thiopenem antibiotic being developed for the treatment of infections caused by multi-drug resistant bacteria
 - Exerts bactericidal activity through inhibition of bacterial cell wall synthesis by binding to penicillin-binding proteins
 - Has potent activity against Enterobacteriaceae, including those with ESBLs or AmpC-type β-lactamases
 - Is available as intravenous and oral formulations.
- During a phase 1 pharmacokinetic clinical study in healthy volunteers, urine was collected and tested for activity against key pathogens.
- The purpose of the present study was to perform an *in vitro* investigation of the urine minimum bactericidal titres for patient urine samples, against two *E. coli* reference quality control (QC) strains, two clinical isolates of *E. coli* and one clinical isolate of *K. pneumoniae*.

METHODS

- Voided urine from 0-2 hours post-dose was collected from 128 subjects who were given sulopenem etzadroxil in a Phase 1 PK study, and a 5 mL specimen from this collection was stored frozen.
- Six isolates were tested: *E. coli* ATCC 25922 (CLSI reference QC control); *E. coli* ATCC 35218 (CLSI β-lactamase inhibitor QC control); *E. coli* 1373629 (ESBL-positive), *E. coli* 1381423 (ESB- negative; DHA-1 AmpC positive), *K. pneumoniae* 1373657 (ESBL-negative; OXA-48 positive) and *P. aeruginosa* ATCC 27853 (CLSI reference QC control; MIC only).
- Standard MIC determinations using sulopenem, imipenem and meropenem were performed in broth microdilution as per CLSI susceptibility testing standards [M07-A10].
 - Isolates were subcultured once on tryptic soya agar plates containing 5% sheep blood from frozen stock.
 - A second subculture was made by diluting a 0.5 McFarland prepared from the first subculture in 100 mL cation-adjusted Mueller Hinton broth (CA-MHB) and incubating it overnight at room temperature without shaking.
 - On the day of testing, the liquid cultures were diluted to a 0.5 McFarland and then diluted further by 10 in a flask containing 100 ml CA-MHB in order to obtain approximately 1 x 10⁷ CFU/mL. The cultures were incubated at 35°C with shaking for about 1 hour, until a 0.6 McFarland was attained (exponential growth).
 - The cultures were then diluted by 50 in commercial pooled human urine to obtain approximately 1 x 10⁶ CFU/mL. This dilution was used as the inoculum and to make the CFU counts for the UBT determination.
 - Panels containing 50 µL of urine samples were diluted 2-fold with 50 µL of inoculum to give a final inoculum of ca. 5x10⁵ CFU/mL. The test plates were incubated at 35°C for 24h and read visually. Thus the dilutions investigated ranged from 1:2 to 1:1024.
 - To determine UBTs, two aliquots of 10 µL were taken from each well of the 96-well plate and spotted onto 40 mL Mueller Hinton agar plates. These plates were allowed to dry and then incubated for 24h at 35 °C. Colonies were counted and UBT was defined as the highest dilution of urine that produced 99.9% kill (3 x log₁₀) of the test inoculum.
- UBT determinations were performed by broth microdilution in line with CLSI guideline M21-A as follows:
 - Isolates were subcultured once on tryptic soya agar plates containing 5% sheep blood from frozen stock.
 - A second subculture was made by diluting a 0.5 McFarland prepared from the first subculture in 100 mL cation-adjusted Mueller Hinton broth (CA-MHB) and incubating it overnight at room temperature without shaking.
 - On the day of testing, the liquid cultures were diluted to a 0.5 McFarland and then diluted further by 10 in a flask containing 100 ml CA-MHB in order to obtain approximately 1 x 10⁷ CFU/mL. The cultures were incubated at 35°C with shaking for about 1 hour, until a 0.6 McFarland was attained (exponential growth).
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RESULTS

Table 1: Bactericidal Activity of Sulopenem in Urine

Organism [Sulopenem MIC]	Sulopenem-etzadroxil fasted (n=32)	Maximum fold-dilution of urine retaining bactericidal activity	
		Sulopenem-etzadroxil fasted (n=32)	Sulopenem-etzadroxil fed (n=31)
<i>E. coli</i> ATCC 25922 [0.015 µg/mL]	32	32	64
<i>E. coli</i> ATCC 35218 [0.015 µg/mL]	128	128	256
<i>E. coli</i> 1373629 [0.03 µg/mL]	256	256	256
<i>E. coli</i> 1381423 [0.12 µg/mL]	16	16	32
<i>K. pneumoniae</i> 1373657 [OXA-48:16 µg/mL]	2	2	4

Table 2: Susceptibility of Test Bacteria to Sulopenem, Imipenem and Meropenem

Isolate tested	Minimum inhibitory concentration (µg/mL)								
	Sulopenem			Imipenem			Meropenem		
	A	B	C	A	B	C	A	B	C
<i>E. coli</i> ATCC 25922	0.015	0.015	0.015	≤0.5	≤0.5	≤0.5	≤0.12	≤0.12	≤0.12
<i>E. coli</i> ATCC 35218	0.015	0.015	0.015	≤0.5	≤0.5	≤0.5	≤0.12	≤0.12	≤0.12
<i>E. coli</i> 1373629	0.03	0.03	0.03	≤0.5	≤0.5	≤0.5	≤0.12	≤0.12	≤0.12
<i>E. coli</i> 1381423	0.12	0.12	0.25	≤0.5	≤0.5	≤0.5	≤0.12	≤0.12	≤0.12
<i>K. pneumoniae</i> 1373657	16	16	8	4	8	4	4	4	4

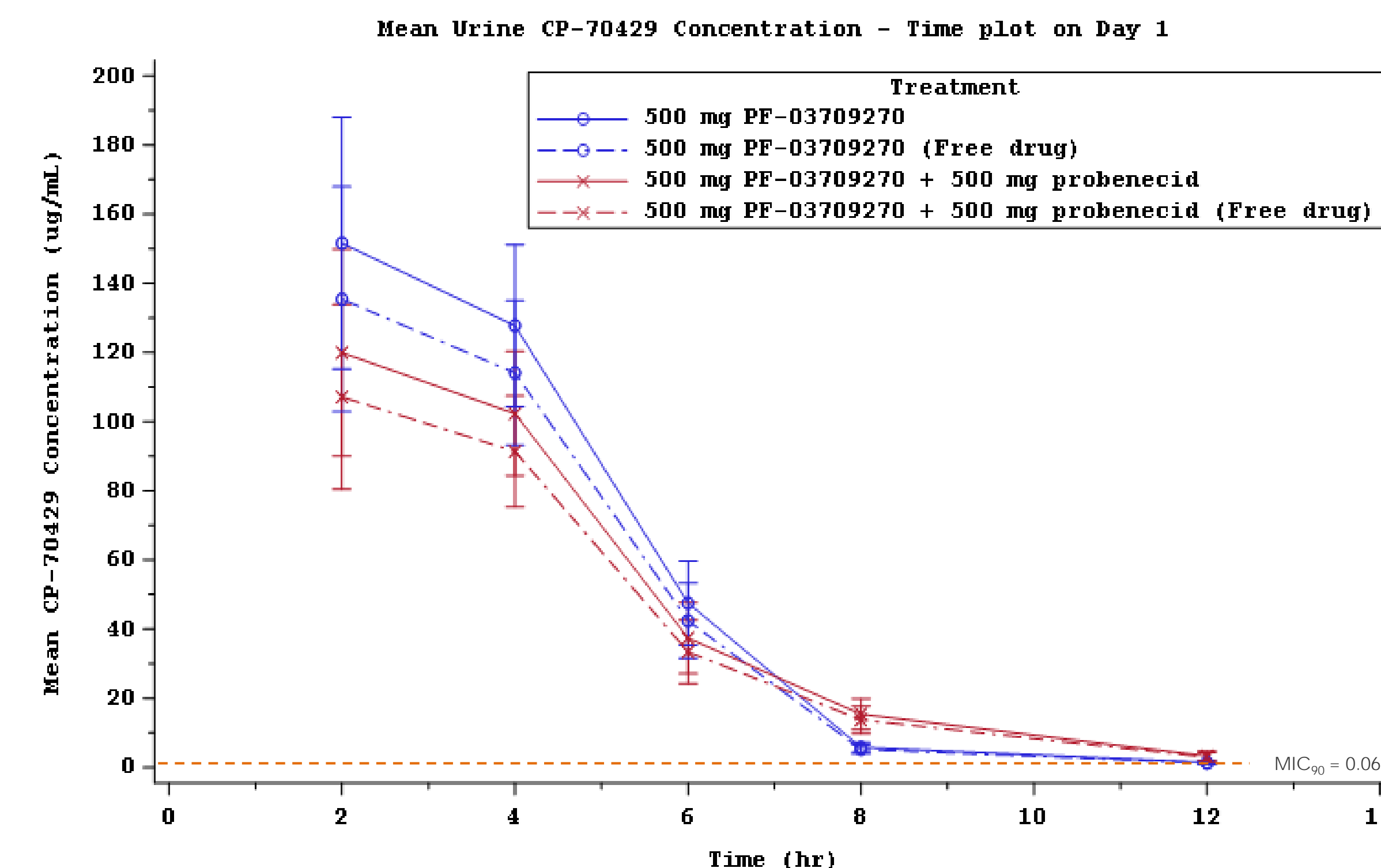
A, B, C; individual MIC values for triplicate tests; Values represent the MIC in µg/mL

Table 3: Concentration of Sulopenem in Urine

Time point	Parameter (µg/mL)	Sulopenem etzadroxil (Fed)		Sulopenem etzadroxil (Fasted)	
		With Probenecid	Without Probenecid	With Probenecid	Without Probenecid
0-2 Hours	N	8	8	8	8
	Mean (SD)	87.1 (81.09)	201.0 (190.22)	152.9 (147.04)	102.2 (60.34)
	Median	55.5	118.5	74.6	91.7
	Min, Max	35.8, 279.0	28.8, 530.0	21.8, 420.0	33.4, 192.0
2-4 Hours	N	8	8	8	8
	Mean (SD)	144.2 (51.89)	156.1 (106.16)	60.5 (66.40)	99.5 (75.44)
	Median	127.5	149.2	39.8	76.3
	Min, Max	82.5, 228.0	32.6, 329.0	21.5, 222.0	51.2, 279.0
4-6 Hours	N	8	8	8	8
	Mean (SD)	46.0 (41.15)	67.1 (58.68)	28.9 (41.75)	28.0 (27.22)
	Median	37.6	44.9	13.2	15.1
	Min, Max	8.5, 141.0	2.6, 174.0	1.7, 129.0	8.9, 81.1
6-8 Hours	N	8	8	7	8
	Mean (SD)	23.0 (20.64)	9.1 (6.66)	6.7 (5.90)	2.6 (1.37)
	Median	17.3	7.7	4.7	2.6
	Min, Max	3.3, 59.5	1.8, 19.9	2.2, 19.6	0.8, 4.3
8-12 Hours	N	8	8	8	8
	Mean (SD)	5.5 (7.79)	2.2 (1.99)	1.1 (0.69)	0.6 (0.39)
	Median	2.5	1.3	1.0	0.5
	Min, Max	1.0, 24.1	0.2, 5.1	0.2, 2.5	0.1, 1.1

RESULTS

Figure 1: Sulopenem Concentration in Urine after Dosing with Sulopenem Etzadroxil (PF-03709270)



Conclusions

- Bactericidal activity against all organisms, including the OXA-48 producing organism, was evident in all urine samples tested indicating potent antimicrobial activity of sulopenem in the urine.
- Sulopenem provided to patients in the fed state was, in most cases, one-doubling dilution more bactericidal relative to those dosed fasted.
- High concentrations of sulopenem were observed in the urine and they remained above the MIC₉₀ of target pathogens for the entire duration of dosing
- These data support further study of sulopenem for the treatment of uUTI.