P1705

In vitro activity of Sulopenem Against Resistant *Neisseria gonorrhoeae*

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ABSTRACT

Background: Sulopenem is a novel thiopenem antibiotic with activity against multidrug resistant Enterobacteriaceae. *In vitro* activity has also been described against wild type *Neisseria gonorrhoeae* with an MIC_{90} of =–0.05 µg/mL (Minamimura, AAC 1993). Given concerns around the effectiveness of existing therapies, the activity of sulopenem was studied against organisms with elevated MICs to commonly used antibiotics.

Methods: Agar dilution testing was performed according to CLSI methodology in cysteine free GC agar. Sixty-four resistant microorganisms (US Centers for Disease Control AR Isolate Bank and Neisseria Reference Lab isolate collections) were selected based on resistance to azithromycin (9 isolates), ciprofloxacin (57 isolates) and ceftriaxone (1 isolate).

Results: Susceptibility of *Neisseria gonorrhea* to Sulopenem in Isolates Resistant to Common Agents

	Sulopenem Mean Inhibitory Concentration (µg/mL)						
	0.03	0.06	0.12	0.25	0.5	1	2
All isolates (N = 64)	7	1	18	13	16	8	1
Ciprofloxacin R	4	1	15	12	16	8	1
Ciprofloxacin S	3		2				
Azithromycin R (MIC ≥ 4 µg/mL)	4	1	4				
Ceftriaxone S	7		18	14	14	8	1
Ceftriaxone NS (MIC = 0.5 µg/mL)			1				
Cefixime NS (MIC = 1 µg/mL)			1				
Cefixime S	3		14	10	10	6	1
Beta-lactamase positive*	4		1				

NS=non-susceptible; R=resistant; S= susceptible

Conclusions: Sulopenem is active *in vitro* against isolates resistant to antibiotics commonly used to treat gonococcal infections. Sulopenem etzadroxil, the oral prodrug of sulopenem, should be evaluated for the treatment of gonorrhea.

INTRODUCTION

- A reduction in susceptibility to antibiotics typically used to treat infections caused by *Neisseria gonorrhoeae* has been identified globally.
- The World Health Organization has called for the development of new antibiotics to treat antibiotic-resistant gonorrhea
- Sulopenem is a thiopenem antibiotic being developed for the treatment of infections caused by multi-drug resistant bacteria
  - Has potent activity against Enterobacteriaceae
    - Including those with ESBLs or AmpC-type β-lactamases, and those that are quinolone non-susceptible
  - Has an intravenous and oral formulation
  - Being developed for the treatment of urinary tract infection and complicated intra-abdominal infection
  - Exerts bactericidal activity through inhibition of bacterial cell wall synthesis by binding to penicillin-binding proteins

METHODS

- Agar dilution testing for sulopenem susceptibility was performed according to the CLSI methodology (cysteine free GC agar and allowing the agar to cool to 50°C before adding sulopenem). [CLSI document M7-A11]
  - A pilot study was performed with a subset of 26 *N. gonorrhoeae* to test the temperature of the agar at the time sulopenem was added to the liquid media prior to pouring the plates, one set made with sulopenem added at 50°C and 60°C. Also, one set of each was made with and without cysteine
- Triplicate testing was performed for a subset of 5 isolates for assessment of reproducibility and the geometric mean MIC rounded to the nearest doubling dilution was used in the MIC distributions
- The 64 microorganisms tested in this study were from the LSI and CDC isolate collections and chosen based on their resistance profiles
  - MIC testing results against ciprofloxacin, cefixime, azithromycin and ceftriaxone was previously performed by CDC or LSI

RESULTS

Table 1. Quality Control Results

Quality control strain	Expected MIC Range (µg/mL)	Pilot Study (µg/mL)				Resistance Study (CF50) (µg/mL)		
		CF50	CF60	C50	C60	1	2	3
Escherichia coli ATCC 25922	0.015-0.06	0.03	0.03	0.06	0.06	0.03	0.03	0.06
Haemophilus influenzae ATCC 49766	0.06-0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25
Neisseria gonorrhoeae ATCC 49266	Not Available	0.12	0.12	0.25	0.25	0.06	0.06	0.06

CLSI M100-S28, based on testing with cation adjusted Mueller Hinton agar
CLSI M100-S28, based on testing with Haemophilus Test Media
CF50 - Cysteine-free GC agar, sulopenem added at 50oC
CF60 - Cysteine-free GC agar, sulopenem added at 60oC
C50 - Cysteine in GC agar, sulopenem added at 50oC
C50 - Cysteine in GC agar, sulopenem added at 60oC

Table 2. Pilot Study Results: sulopenem dilution difference compared to cysteine free agar - sulopenem added at 50°C

Method	-2	-1	0	1	2	3	n
Cysteine free agar – Sulopenem added at 60°C		1	22	3			26
Agar containing Cysteine - Sulopenem added at 50°C				7	19		26
Agar containing Cysteine - Sulopenem added at 60°C				2	22	2	26

Note: Growth on cysteine free agar was recorded as lighter than growth on cysteine containing agar

RESULTS

Table 3: Line Listing of MIC Results

Isolate #	Azithromycin (µg/mL)	Ceftriaxone (µg/mL)	Ciprofloxacin (µg/mL)	Sulopenem (µg/mL)
1	0.5	0.06	8	0.12
2	0.12	0.03	>1	1
3	0.25	0.008	>1	1
4	8	0.015	>1	0.06
5	0.12	0.03	>1	0.03
6	0.12	0.015	>1	0.12
7	0.12	0.008	>1	0.03
8	0.12	0.03	>1	0.25
9	0.25	0.06	>1	0.5
10	0.12	0.008	>1	0.12
11	8	0.008	>1	0.03
12	0.12	0.03	>1	0.5
13	0.5	0.015	>1	0.03
14	0.5	0.06	>1	0.25
15	0.5	0.03	>1	0.25
16	0.25	0.03	>1	0.25
17	0.5	0.015	>1	0.5
18	0.25	0.004	>1	0.5
19	8	0.004	>1	0.12
20	0.5	0.5	0.015	0.12
21	1	0.125	8	0.25
22	8	0.008	0.015	0.03
23	0.5	0.06	16	0.25
24	1	0.06	16	0.5
25	1	0.06	16	0.25
26	0.5	0.06	16	0.25
27	0.5	0.06	16	0.25
28	1	0.125	16	0.50
29	0.5	0.03	16	0.50
30	1	0.06	8	2
31	0.5	0.125	16	0.12
32	16	0.008	0.015	0.03
33	1	0.06	16	0.12
34	8	0.008	0.015	0.12
35	0.5	0.06	16	1
36	256	0.03	0.015	0.12
37	0.5	0.06	16	0.12
38	0.5	0.06	16	0.12
39	0.5	0.06	16	0.5
40	0.5	0.125	16	0.5
41	2	0.03	4	0.5
42	1	0.06	16	0.5
43	1	0.125	16	0.25
44	1	0.06	16	0.25
45	1	0.06	8	0.5
46	1	0.06	16	0.5
47	0.5	0.03	8	0.5
48	4	0.03	16	0.12
49	1	0.06	16	0.12
50	0.5	0.125	16	1
51	0.5	0.125	8	1
52	16	0.008	0.015	0.03
53	0.5	0.125	16	1
54	0.5	0.06	16	0.12
55	0.5	0.06	16	0.12
56	0.5	0.06	16	0.5
57	0.5	0.06	16	1
58	1	0.06	16	0.25
59	1	0.06	16	0.25
60	0.25	0.06	16	0.12
61	1	0.06	16	0.12
62	0.5	0.06	16	0.12
63	0.5	0.06	16	0.5
64	0.5	0.125	16	1

Table 4: Summary of Sulopenem MIC Results Against Drug Resistant *N. gonorrhoeae*

	Sulopenem Mean Inhibitory Concentration (µg/mL)						
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Cefixime S	3		14	10	10	6	1
Beta-lactamase positive*	4		1				

NS=non-susceptible; R=resistant; S= susceptible; * 3 additional isolates run in separate pilot study

CONCLUSIONS

- Sulopenem demonstrates *in vitro* activity against this challenge set of *N. gonorrhoeae*
- An isolate non-susceptible to ceftriaxone and cefixime had a sulopenem MIC of 0.12 µg/mL
- Isolates resistant to azithromycin or ciprofloxacin had an MIC to sulopenem of ≤ 0.12 µg/mL
- The addition of sulopenem to the liquid GC agar at 60°C did not effect the sulopenem MIC though the addition of cysteine increased the MIC results by 1-3 dilutions; cysteine-free media against *N. gonorrhoeae* should be utilized
- Additional surveillance studies should be performed and, if consistent, clinical studies with sulopenem in the treatment of gonococcal infection should be considered

ITERUM therapeutics