Evaluation of Bactericidal Activity, Post-Antibiotic Effect and Sub-Inhibitory Effect of Gepotidacin Against Various Gram-Negative and Gram-Positive Isolates

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Introduction

Gepotidacin (GSK2140944) is a novel, bactericidal, first in class triazaacenaphthylene antibiotic in clinical development for the treatment of gonorrhea and uncomplicated urinary tract infection (acute cystitis).

Gepotidacin selectively inhibits bacterial DNA replication by a distinct mechanism of action, which confers *in vitro* activity against most strains of target pathogens, such as *E. coli, S. saprophyticus* and *N. gonorrhoeae,* including those resistant to current antibiotics.

This study investigated the *in vitro* bactericidal activity, post-antibiotic effect (PAE), and sub-inhibitory MIC effect (PAE-SME) of gepotidacin and levofloxacin using time-kill assay methods.

Materials and Methods

The activities of gepotidacin and levofloxacin were evaluated against 3 clinical isolates from each of the following species: *C. freundii*, *E. cloacae* species complex, *K. aerogenes*, *K. pneumoniae*, *P. mirabilis*, *P. rettgeri*, *E. faecalis*, and *S. saprophyticus* collected in 2019.

The organisms tested included a mixture of wild-type, extended-spectrum beta-lactamase-producing, and fluoroquinolone-resistant phenotypes.

Baseline MIC values were determined in triplicate using the Clinical and Laboratory Standards Institute (CLSI) M07Ed11 [2018] broth microdilution (BMD) method with cation-adjusted Mueller-Hinton broth (CAMHB).

Time-kill kinetic studies

- Completed in CAMHB containing the antimicrobial agent at ¼x, ½x, 1x, 2x, 4x, or 10x the MIC.
- Samples were collected for CFU/mL determination at time 0 hours (T0), T2, T4, T8, and T24.
- Viable cell counts were determined after 24 hours incubation at 35°C.
- Bactericidal activity was defined as a 3-$\log_{10}$ decrease in CFU/mL after 24 hours exposure.

Post antibiotic effect (PAE) studies

- Cultures were exposed at 1x, 5x, and 10x the baseline broth microdilution MIC.
- Samples were collected for CFU/mL determination at time 0 hours (T0), T2, T4, T8, and T24.

PAE-sub-MIC effect (PAE-SME) studies

- Cultures were initially exposed for 1-2 hours at 5x MIC, followed by ¼x or ½x MIC re-exposure.
- Samples were collected hourly for viable cell counting (CFU/mL) for up to 9 hours.

PAE and PAE-SME interpretation

- PAE and PAE-SME are defined as the difference (treated vs untreated cultures) in time required for CFUs/mL to increase 1-$\log_{10}$ post exposure.
- PAE and PAE-SME values interpreted as short (≤1 hour), modest (>1-4 hours), or extended (>4 hours).

Disclosures

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Gepotidacin displayed concentration-dependent activity in bactericidal, PAE, and PAE-SME time-kill experiments.

Gepotidacin was bactericidal by 8 hours against 66% and 58% of isolates when tested at 4x and 10x MIC, respectively.

In general, modest PAEs (>1-4 hour) and extended PAE-SMEs (>4 hours) for gepotidacin were observed.

Table 1 Summary of time-kill, PAE, and PAE-SME results for isolates exposed to various gepotidacin concentrations.

Isolate No.	Organism	Key phenotype	Gepotidacin MIC (mg/L)	$\log_{10}$ reduction in CFUs at T24[a]						PAE (hours)[b]			PAE-SME (hours)[b]	
				1/4x[c]	1/2x	1x	2x	4x	10x	1x	5x	10x	5x→¼x	5x→½x
1091286	*C. freundii* sc	WT	1	-2.2[d]	-1.2	-1.6	-0.8	4.4[c]	5.7	0.2	1.0	2.0	3.8	>6.5
1130512	*C. freundii* sc	ESBL	2	-1.6	-1.1	2.1	2.8	2.0	1.4	1.7	2.6	3.1	>6.4	>6.4
1116313	*C. freundii* sc	FQ-R	16	-1.7	-1.3	-0.4	5.9	3.6	3.7	2.0	2.4	3.0	>6.9	>6.9
1092886	*E. cloacae* sc	WT	4	-2.0	-1.8	-1.5	1.9	2.9	3.1	0.6	1.9	2.6	5.1	6.1
1092279	*E. cloacae* sc	ESBL	4	-1.7	-2.2	-1.2	-0.9	5.7	5.7	2.0	3.0	3.1	6.4	>7.2
1127328	*E. cloacae* sc	FQ-R	8	-1.0	-0.1	2.1	4.1	4.4	4.4	1.6	3.4	6.1	>7.0	>7.0
1089847	*K. aerogenes*	WT	2	-1.2	0.2	0.2	4.4	5.7	5.7	1.3	2.9	3.0	5.3	>6.8
1089299	*K. aerogenes*	ESBL	2	-1.0	1.2	1.5	2.9	1.8	1.1	-0.1	0.7	1.7	3.6	>6.5
1092937	*K. aerogenes*	FQ-R	8	-0.1	0.6	4.6	4.3	4.1	4.6	1.9	4.5	4.8	>7.0	>7.0
1098581	*K. pneumoniae*	WT	4	-2.1	-1.4	-2.4	-0.6	3.9	5.9	0.7	2.3	2.8	4.5	>7.0
1130299	*K. pneumoniae*	ESBL	4	-1.7	-0.9	-0.2	0.2	3.1	3.5	0.7	1.0	1.5	3.3	4.7
1124085	*K. pneumoniae*	FQ-R	32	-1.6	-0.4	0.7	3.0	4.6	5.9	1.3	>6.6	>6.6	>6.6	>6.6
1091952	*P. mirabilis*	WT	16	-1.0	-0.2	0.1	0.8	5.7	5.7	0.9	3.0	>6.7	>6.7	>6.7
1106732	*P. mirabilis*	ESBL	0.5	-1.1	-1.7	-0.8	0.3	5.2	5.2	0.8	1.4	2.2	2.2	3.1
1093555	*P. mirabilis*	FQ-R	8	-1.7	-2.6	-2.3	0.7	3.8	5.1	1.0	2.4	3.9	3.7	>6.8
1089529	*P. rettgeri*	WT	4	-1.1	0.8	1.6	3.5	4.7	4.4	0.6	0.3	>6.4	>6.4	>6.4
1090192	*P. rettgeri*	ESBL	4	-0.6	2.6	6.1	6.1	6.1	6.1	0.7	0.7	2.3	>6.4	>6.4
1118004	*P. rettgeri*	FQ-R	4	-0.9	-0.5	1.8	3.5	3.6	5.7	1.4	2.2	2.7	>6.6	>6.6
1103850	*E. faecalis*	WT	0.25	-3.1	-2.5	-1.2	3.9	3.3	2.7	1.2	2.7	3.4	4.4	>7.0
1111210	*E. faecalis*	WT	1	-1.9	-1.2	1.6	3.6	4.2	3.7	-0.3	1.2	2.0	3.8	>5.0
1097863	*E. faecalis*	FQ-R	1	-2.1	-1.2	0.9	2.8	2.6	2.7	1.4	2.4	3.2	>5.9	>5.9
1106006	*S. saprophyticus*	WT	0.06	-2.6	-2.6	1.7	5.5	5.5	5.5	2.0	4.3	5.2	>6.1	>6.1
1113726	*S. saprophyticus*	WT	0.06	-2.4	-1.5	1.8	5.6	5.6	5.6	1.7	3.6	3.7	>6.1	>6.1
1129086	*S. saprophyticus*	WT	0.06	-3.0	-2.6	-1.8	0.9	5.7	5.7	0.9	2.7	3.1	4.1	>5.7

sc, species complex; WT, wild type; ESBL, extended spectrum β-lactamase; FQ-R, fluoroquinolone resistant

[a] Shaded values represent a ≥3-log10 decrease in CFUs (bactericidal) compared to starting inoculum.

[b] Shaded values represent modest (>1-4 hours) or extended (>4 hours) interpretations.

[c] Gepotidacin concentration (relative to MIC)

[d] Negative values indicate increased growth compared to untreated control.

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Results

Among the 18 gram-negatives, gepotidacin displayed bactericidal activity in a concentration-dependent manner (Table 1).

- 1x MIC: 2 isolates (11%)
- 2x MIC: 8 isolates (44%)
- 4x MIC: 15 isolates (83%)
- 10x MIC: 16 isolates (89%)

Against the 6 gram-positives, gepotidacin displayed bactericidal activity.

- 2x MIC: 4 isolates (67%)
- 4x MIC: 5 isolates (83%)
- 10x MIC: 4 isolates (67%)

The time required to achieve a ≥3-$\log_{10}$ decrease in CFU/mL ranged from 1-24 hours for both gram-negative and gram-positive isolates.

Gepotidacin PAEs were concentration dependent (Table 1).

- 1x MIC exposure: PAEs were short (12 isolates, 50%) or modest (12 isolates, 50%)
- 5x MIC exposure: PAEs were predominately modest (16, 67%)
- 10x MIC exposure: PAEs were modest (18, 75%) to extended (6, 25%)

Gepotidacin displayed extended PAE-SMEs (Table 1).

- When challenged with 0.5x MIC after 5x MIC exposure, 21 (88%) isolates failed to increase 1-$\log_{10}$ in CFUs (PAE-SME of >5.0 to >7.2 hours).
- When challenged with 0.25x MIC after 5x MIC exposure, 12 (50%) isolates failed to increase 1-$\log_{10}$ in CFUs (PAE-SME of >5.9 to >7.0 hours).
- The average PAE-SME observed at 0.5 and 0.25x MIC among the isolates with on-scale results was 4.6 and 4.2 hours, respectively.

Levofloxacin was also tested in this study and showed similar time-kill, PAE, and PAE-SME results when compared to gepotidacin (data not shown).

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