An Evaluation of Tebipenem *In Vitro* Activity Against a Panel of *Pseudomonas aeruginosa* Isolates with Efflux, AmpC, and OprD Mutations

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INTRODUCTION

- Tebipenem pivoxil hydrobromide is an orally bioavailable prodrug of tebipenem, a novel oral carbapenem with activity against Gram-positive and -negative bacteria. Tebipenem pivoxil hydrobromide is currently being developed for the treatment of patients with complicated urinary tract infections.
- Given that *Pseudomonas aeruginosa* has become one of the most common pathogens associated with nosocomial infections [1], a series of susceptibility studies for tebipenem were undertaken.
- As described herein, the susceptibility studies undertaken were designed to evaluate the activity of tebipenem against a panel of *P. aeruginosa* isolates known to have alterations in efflux pumps, outer-membrane porins, and expression of AmpC beta-lactamase enzymes compared to a subset of wild-type organisms.

METHODS

Antimicrobial Agent and Challenge Isolates

- A panel of 20 *P. aeruginosa* isolates was obtained from Spero Therapeutics, Inc. (Cambridge, MA), Dr. Poole (Ontario, CA) [2, 3], the Institute for Clinical Pharmacodynamics, Inc. internal collection (Schenectady, NY), and JMI Laboratories (North Liberty, IA).
- The Clinical Laboratory Standards Institute (CLSI) internal control isolates were purchased from the American Type Culture Collection (Manassas, VA).
- Tebipenem was provided by the Sponsor (Spero Therapeutics, Inc., Cambridge, MA) while meropenem, ertapenem, levofloxacin, tetracycline and carbenicillin were purchased from Sigma-Aldrich Corporation, LLC (St. Louis, MO).

Susceptibility Studies

- Each *P. aeruginosa* isolate in the challenge panel was subjected to tebipenem and five other challenge compounds (meropenem, ertapenem, levofloxacin, tetracycline, and carbenicillin) in triplicate, using standard agar dilution methodologies [4] in order to determine minimum inhibitory concentration (MIC) values.
- Each MIC value was determined in triplicate over a two-day period and reported as the modal value.
- *P. aeruginosa* ATCC 27853 was used as an internal control.

RESULTS

- As shown in **Table 1**, tebipenem MIC values ranged from 0.25 to > 64 mg/L for the 20 *P. aeruginosa* isolates evaluated.
- These MIC ranges were similar to those found for meropenem and ertapenem, the MIC values for which ranged from ≤ 0.03 to 16 mg/L and ≤ 0.25 to 128 mg/L, respectively.

Table 1. Modal agar-dilution MIC values for all compounds evaluated against the panel of 20 *P. aeruginosa* challenge isolates

P. aeruginosa isolate	Known resistance mechanisms	Modal agar MIC (mg/L)					
		Tebipenem	Meropenem	Ertapenem	Levofloxacin	Tetracycline	Carbenicillin
ATCC 27853 (SPT-18)	Wild type	2	0.25	4	1	8	32
ATCC 12055 (SPT-19)	Parent strain to SPT-20	4	0.5	8	0.5	8	8
ATCC 35151 (SPT-20)	Hyper-susceptible to antibiotics	0.25	≤ 0.03	≤ 0.25	≤ 0.06	0.5	≤ 0.125
K767 (SPT-24)	PA01 prototroph, parental strain of SPT-26 through SPT-32	2	0.5	8	0.25	16	32
K1523 (SPT-25)	ΔmexB[a]	1	0.125	4	0.125	8	0.5
K2958 (SPT-26)	ΔmexCD-oprJ[a]	2	0.5	8	0.25	8	32
K1525 (SPT-27)	ΔmexX[a]	2	0.5	8	0.25	8	32
K2733 (SPT-29)	ΔmexAB-oprM, ΔmexCD-oprJ, ΔmexEF-oprN, ΔmexXY[a]	1	0.125	4	≤0.06	0.5	0.5
K1455 (SPT-30)	nalB[b]	8	1	16	2	64	> 128
K1536 (SPT-31)	nfxB[b]	2	0.5	8	2	16	32
K2415 (SPT-32)	ΔmexZ[b]	2	0.5	8	0.5	16	16
K2153 (SPT-33)	WT clinical strain	4	0.5	4	0.5	16	32
K2892 (SPT-34)	ΔmexF[a]	4	0.5	4	0.5	16	32
K2376 (SPT-35)	ΔmexS[b]	4	0.5	4	1	16	8
2881	PA01 derepressed AmpC	2	0.5	8	0.25	8	64
3614	Oprd loss, moderate AmpC	8	2	32	≤ 0.06	4	128
3616	derepressed AmpC	4	1	8	16	16	64
3653	Oprd loss, Elevated AmpC	> 64	16	128	2	64	> 128
3690	Oprd loss	32	8	64	16	16	64

a. Isolates that were efflux deficient.
b. Isolates that demonstrated increased efflux.

RESULTS

- The hyper-susceptible isolate ATCC 35151 was the most susceptible to the carbapenems, with MIC values ranging from ≤ 0.03 to 0.25 mg/L. These MIC values were at least 16-fold lower than the parent isolate, ATCC 12055.
- The deletion of MexB decreased the carbapenem MIC values by 2 to 4x while the deletion of MexCD-OprJ and MexXY did not result in such a decrease.
- Simultaneous deficiencies in MexAB-OprM, CD-OprJ, EF-OprN, and XY did not decrease MIC values in comparison to the deletion of MexAB-OprM alone.
- Isolates 2881 and 3616, possessing a derepressed AmpC, showed no significant elevation in MIC value compared to wild-type isolates, while those known to have lost OprD, isolates 3614 and 3690, resulted in carbapenem MIC values that were increased by 4 to 32x.
- The combined loss of OprD and overexpression of AmpC resulted in the highest carbapenem MIC values (≥ 16 mg/L).
- Levofloxacin, tetracycline, and carbenicillin MIC values ranged from ≤ 0.06 to 16 mg/L, 0.5 to 64 mg/L, and ≤ 0.125 to > 128 mg/L, respectively.
- All MIC values determined for the internal control isolate ATCC 27853 were within CLSI reported values [5].

CONCLUSIONS

- These data provide insight into the effect that alterations in efflux pump, outer-membrane porins, and expression of AmpC enzymes have on the activity of tebipenem and comparison antibiotics.
- The activity of tebipenem against *P. aeruginosa* is similar to that observed for ertapenem. Therefore, like ertapenem, *P. aeruginosa* is not being considered as a target pathogen for tebipenem.

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