MK-3866, a Metallo-β-Lactamase Inhibitor, Is Not Subject to Efflux in *Pseudomonas aeruginosa*

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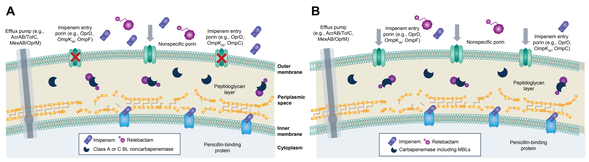
Background

- Carbapenem-resistant *Pseudomonas aeruginosa* is a serious threat to human health and has been identified as a critical priority pathogen for which current therapeutic options are insufficient[1,2]
- In 2020, coincident with the COVID-19 pandemic, multidrug-resistant *P. aeruginosa* cases in hospitals rose significantly[3]
- *P. aeruginosa* have efflux systems that can impair the activity of antibacterial agents[4,5]
  - They are predominantly tri-partite resistance-nodulation-cell division type and MexAB-OprM pump is an example[4]
  - Other common resistance mechanisms include carbapenemase production or concurrent decreased OprD expression with overexpression of β-lactamases[6] (**Figure 1**)
- Imipenem/relebactam is a β-lactam/β-lactamase inhibitor combination that inhibits Ambler class A and C enzymes and is not subject to efflux[6,7]
- Metallo-β-lactamases (MBLs) (eg, imipenem MBL-1 [IMP-1], Verona integron-encoded MBL [VIM-1 and VIM-2], and New Delhi MBL [NDM]) are class B enzymes that use 1 or more active-site zinc ions to facilitate hydrolysis of β-lactam antibacterial agents[8]
  - They are not inhibited by approved β-lactamase inhibitor combinations[9,10]
  - Effective treatment for infections caused by MBL-producing gram-negative bacteria is a significant unmet medical need worldwide
- MK-3866 is a small-molecule pan-MBL inhibitor being assessed for use with β-lactam/β-lactamase inhibitor combinations to restore activity against resistant MBL-expressing gram-negative bacteria, such as *P. aeruginosa*
- The objectives of the study are as follows:
  - To determine the potential of MBL inhibitors to be effluxed from *P. aeruginosa*
  - To characterize MK-3866 and analogs for potentiation of imipenem/relebactam or cefepime/relebactam in isogenic strain pairs of efflux wild-type (WT) and multiply efflux–deleted (MED) strains of *P. aeruginosa*

Figure 1. Mechanisms of Efflux in *Pseudomonas aeruginosa* Strains Expressing Resistance Mechanisms: (A) Porin Loss and Class A or C β-lactamases (Noncarbapenemase); (B) Carbapenemases, Including Metallo-β-Lactamases



BL, β-lactamase; MBLs, Metallo β-lactamases

Methods

Isogenic *P. aeruginosa* Efflux Isolates

- Isogenic *P. aeruginosa* efflux WT (MB5919) and MED (MB5890) strains have been described previously[5,6]
- The background of this strain pair carried an *nfx*C mutation (Cumbre Pharmaceuticals, Dallas, TX, USA)
  - WT strain was PAO1 *nfx*C
  - MED strain was PAO1 *nfx*C Δ(mexAB-oprM) Δ(mexCD-oprJ) Δ(mexXY) Δ(mexJKL) Δ(mexHI-opmD) Δ(opmH)[6]
- An 8-base-pair mutation in the strains resulted in overexpression of the MexEF-OprN efflux pump[6]
  - Resulted in reduction in expression of the imipenem entry porin OprD, which raised the minimum inhibitory concentration (MIC) to imipenem slightly[11]

Bacterial Strain Production

Production of VIM-1– and VIM-2–Expressing Plasmids

- Genes VIM-1 from plasmid C27756 and VIM-2 from plasmid C29080, and cloning vector pFlp2,[8] were amplified using polymerase chain reaction (PCR) with primer pairs P1/P2, P3/P4, and P5/P6 (**Table 1**)
- VIM-1 and VIM-2 were then cloned into the pFlp2 vector[12]
  - Transformants were checked for pFlp-VIM-1 or pFlp-VIM-2 using PCR with primer pairs P7/P8 and P9/P10 (**Table 1**), respectively

Construction of Efflux +/− Isogenic IMP-1–Expressing P. aeruginosa

- IMP-1–expressing plasmid was isolated from *P. aeruginosa* strain CL 5673
- Strains MB5890 and MB5919 were transformed with IMP-1 plasmid by electroporation
- MB5919 cells were selected on cation-adjusted Mueller Hinton agar (CAMHA) containing ceftazidime 16 µg/mL
- MB5890 cells were selected on CAMHA containing ceftazidime 4 µg/mL
- Transformants were checked for IMP-1 plasmids using primer pair P11/P12

SLICE Susceptibility Testing

- Antibacterial agents (4 µL of a 25× concentrate) and a fixed final concentration of relebactam 4 µg/mL were added to each well of a 96-well round-bottomed polystyrene assay plate
- Susceptibility testing was performed with fixed final imipenem 2 µg/mL or cefepime 8 µg/mL using the Clinical and Laboratory Standards Institute (CLSI) breakpoint for each[13]
- Titration of the MBL inhibitors was carried out in a stock plate of 100× in DMSO and 1 µL was added to each well of the assay plate and mixed
- Colonies were picked from overnight blood agar plate cultures of the test organism and suspended in 1 mL of sterile saline to yield a culture at ~0.5 McFarland or an optical density of 600 nm = 0.1
- Cultures were then diluted 400-fold into fresh 1.05× cation-adjusted Mueller Hinton broth to yield ~5.5 × 10^5 colony-forming units/mL, 94 µL were added to each assay plate. Plates were incubated at 37°C for 18 to 22 hours and read for growth inhibition visually
- The amount of MBL inhibitor required to restore susceptibility to either antibacterial agent (imipenem or cefepime) in efflux isogenic strains was assessed

Table 1. Primers Used in Study

Primer name	Template	Primer sequence
P1	VIM-1	TACTAAGGAGGTTGTATGTTAAAAGTTATTAGTAG
P2	VIM-1	TGCTTAAATGCGTACTTACTCGGCGACTGAGCGATTTT
P3	VIM-2	TACTAAGGAGGTTGTATGTTCAAACTTTTGAGTAAG
P4	VIM-2	TGCTTAAATGCGTACTTACTCAACGACTGAGCGATT
P5	pFlp2	TAAGTACGCATTTAAGCATAAACACGC
P6	pFlp2	CATACAACCTCCTTAGTACATGCAACC
P7	pFlp-VIM1	TTGGTCTACATGACCGCGTCTGTCA
P8	pFlp-VIM1	TAGACCGTGCCCGGGAATGA
P9	pFlp-VIM2	TGGTCTATTTGACCGCGTCTATCA
P10	pFlp-VIM2	GACTGAGCGATTTGTGTGCG
P11	IMP-1	TTTTTGTTTTGCAGCATTGC
P12	IMP-1	TGCTTGGTTTTGATGGTTTT

IMP-1, imipenem metallo-β-lactamase-1; VIM, Verona integron–encoded metallo-β-lactamase.

Results

Characterization of MED Strain Pairs With Selected Antibacterial Agents

- Neither imipenem nor cefepime is effluxed appreciably whether included alone or with relebactam; relebactam is also not subject to efflux
- Chloramphenicol and ciprofloxacin were both highly effluxed
  - ≥128-fold differential in MIC
- For *P. aeruginosa* expressing IMP-1, VIM-1, and VIM-2, MIC values of the β-lactam antibacterial agents increased, but no increase was detected for the non–β-lactam antibacterial agents chloramphenicol and ciprofloxacin (**Table 2**)
  - No meaningful efflux of imipenem, cefepime, imipenem/relebactam, or cefepime/relebactam was observed

Assessment of Efflux Potential of MBL Inhibitors

- The efflux isolate pairs were tested with potential MBL inhibitors in the presence of CLSI breakpoint[13] concentrations of either imipenem (Table 3) or cefepime (Table 4), both with a fixed concentration of relebactam 4 µg/mL
- MIC values for the combination of imipenem/relebactam with MK-3866 or cefepime/relebactam with MK-3866 do not vary more than 2- to 4-fold between efflux WT and MED strains of each pair (**Tables 3 and 4**)

Table 2. Characterization of Efflux Mutant Strain Pairs in the SLICE Assay[a]

Antibacterial agent	MB5919	MB5890	Parent	MB9798	MB9799	IMP-1	MB9861	MB9862	VIM-1	MB9968	MB9969	VIM-2
	Efflux+	Efflux-	Efflux ratio	Efflux+ IMP1	Efflux- IMP1	Efflux ratio	Efflux+ pFlp-VIM1	Efflux- pFlp-VIM1	Efflux ratio	Efflux+ pFlp-VIM2	Efflux- pFlp-VIM2	Efflux ratio
Imipenem	4	1	**4**	32	16	**2**	64	32	**2**	>64	>64	**ND**
Cefepime	0.25	0.25	**1**	>64	>64	**ND**	>64	>64	**ND**	16	16	**1**
Chloramphenicol	>64	1	**≥128**	>64	1	**≥128**	>64	1	**≥128**	>64	1	**≥128**
Ciprofloxacin	1	<0.008	**≥128**	1	<0.008	**≥128**	1	<0.008	**≥128**	1	<0.008	**≥128**
Imipenem and relebactam	0.5	1	**0.5**	32	16	**2**	>64	32	**≥4**	>64	>64	**ND**
Cefepime and relebactam	0.25	0.12	**2**	>64	>64	**ND**	>64	>64	**ND**	16	16	**1**

IMP-1, imipenem metallo-β-lactamase 1; ND, not determinable; VIM, Verona integron–encoded metallo-β-lactamase.
[a]SLICE assay was performed using imipenem 2 µg/mL or cefepime 8 µg/mL with relebactam fixed at 4 µg/mL.

Table 3. Assessment of Efflux Potential in the SLICE Assay[a]: Analog Concentrations Required to Restore Susceptibility to Imipenem in the Presence of Relebactam (MK-7655)

Antibacterial analog	Concentration of metallo-β-lactamase inhibitor to restore susceptibility to imipenem in the presence of relebactam (µg/mL)								
	MB9798	MB9799	IMP-1	MB9861	MB9862	VIM-1	MB9968	MB9969	VIM-2
	MB5919 - IMP-1 WT	MB5890 - IMP-1 MED	Efflux ratio	MB5919 - pFlp-VIM1 WT	MB5890 - pFlp-VIM1 MED	Efflux ratio	MB 5919 - pFlp-VIM2 WT	MB5890 - pFlp-VIM2 MED	Efflux ratio
MK-3866	0.12	0.125	**1**	0.25	0.5	**0.5**	0.25	0.25	**1**
Compound A	4	0.5	**8**	>32	1	**≥64**	>32	2	**≥32**
Compound B	>32	0.5	**≥128**	>32	0.5	**≥128**	32	0.25	**128**
Compound C	16	1	**16**	8	1	**8**	16	2	**8**
Compound D	16	1	**16**	8	1	**8**	16	2	**8**
Compound E	32	0.5	**64**	2	1	**2**	1	0.5	**2**

IMP-1, imipenem metallo-β-lactamase 1; VIM, Verona integron–encoded metallo-β-lactamase.
[a]SLICE assay was performed using imipenem at 2 µg/mL and relebactam fixed at 4 µg/mL.

Table 4. Assessment of Efflux Potential in the SLICE Assay[a]: Analog Concentrations Required to Restore Susceptibility to Cefepime in the Presence of Relebactam

Antibacterial analog	Concentration of metallo-β-lactamase inhibitor to restore susceptibility to imipenem in the presence of relebactam (µg/mL)								
	MB9798	MB9799	IMP-1	MB9861	MB9862	VIM-1	MB9968	MB9969	VIM-2
	MB5919 - IMP-1 WT	MB5890 - IMP-1 MED	Efflux ratio	MB5919 - pFlp-VIM1 WT	MB5890 - pFlp-VIM1 MED	Efflux ratio	MB 5919 - pFlp-VIM2 WT	MB5890 - pFlp-VIM2 MED	Efflux ratio
MK-3866	0.12	0.25	**0.5**	0.25	0.5	**0.5**	<0.03	0.25	**0.12**
Compound A	1	0.5	**2**	32	1	**32**	0.125	0.25	**0.5**
Compound B	>32	0.5	**≥128**	>32	0.5	**≥128**	0.06	0.125	**0.48**
Compound C	8	1	**8**	4	2	**2**	0.125	0.25	**0.5**
Compound D	8	1	**8**	2	2	**1**	0.125	0.5	**0.25**
Compound E	16	0.5	**32**	0.5	1	**0.5**	<0.03	0.25	**0.12**

IMP-1, imipenem metallo-β-lactamase 1; VIM, Verona integron–encoded metallo-β-lactamase.
[a]SLICE assay was performed using cefepime at 8 µg/mL with relebactam fixed at 4 µg/mL.

Conclusions

- The SLICE susceptibility assay facilitated characterization of efflux in isogenic strain pairs of efflux WT and MED strains of *P. aeruginosa* expressing IMP-1, VIM-1, or VIM-2 MBLs
- The concentration of MK-3866 needed to restore imipenem/relebactam or cefepime/relebactam did not differ appreciably between WT or MED strains
  - MK-3866 was not subject to efflux
  - Analogs of MK-3866 were subject to efflux to varying degrees, from nominal to extreme, dependent on both the enzyme expressed as well as the partner antibacterial agent
- Cefepime is subject to efflux in MexXY-overproducing isolates[14]
  - A limitation of this assay is that it does not address the consequences of overexpression of MexXY
- MK-3866 is a promising pan-MBL inhibitor that may act to restore the activity of imipenem/relebactam or cefepime/relebactam in MBL–expressing gram-negative pathogens

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Disclosures

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