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Nacubactam inhibits class A β-lactamases

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ABSTRACT

Background: Nacubactam, formerly RG6080 and OP0595, is a bridged diazabicyclooctane (DBO) that inactivates class A and class C ß-lactamases. Unlike avibactam, the DBO that is approved for use in combination with ceftazidime, nacubactam also inhibits penicillin binding proteins (i.e., PBP2) in Enterobacteriaceae. We set out to determine the effectiveness of meropenem-nacubactam against *Klebsiella pneumoniae* clinical strains and to elucidate the structure-function relationships.

Methods: Minimal inhibitory concentration (MIC) measurements using broth microdilution according to Clinical and Laboratory Standards Institute for meropenem (MERO) ± nacubactam (fixed concentration of 4 mg/L or fixed 1:1 ratio) was performed on 44 clinical *K. pneumoniae* strains harboring KPC-2 or KPC-3 and 47 isogenic *Escherichia coli* strains harboring *bla* genes encoding *K. pneumoniae* carbapenemase (KPC) variants with single amino acid substitutions in residues that are involved in catalysis. IC_{50}s for KPC-2 and the K234R variant were determined on periplasmic extracts with varying concentrations of nacubactam using nitrocefin as a reporter substrate. Inhibition kinetics and timed electrospray-ionization mass spectrometry was performed using purified protein with avibactam and nacubactam.

Results: The MERO combinations with either 4 mg/L or a 1:1 ratio of nacubactam effectively lowered the MERO MICs of *K. pneumoniae* strains. Similarly, all isogenic *E. coli* strains expressing bla_{KPC-2} variants were susceptible to the MERO-nacubactam combinations based on the breakpoint of MERO. The strains harboring P104K and K234R had slightly elevated MERO-nacubactam MICs relative to wild-type but did not have corresponding increases in MERO MICs. The pBR322-K234R strain had a 4-fold lower MERO MIC than pBR322-KPC-2. The nacubactam IC_{50} of cell extracts containing the K234R variant is 781 µM, which is 17-fold higher than that for KPC-2 (46 µM). Both avibactam and nacubactam inhibit KPC-2 more efficiently than the K234R variant.

Conclusion: MERO-nacubactam is an effective ß-lactam ß-lactamase inhibitor combination for *Enterobacteriaceae* with KPC ß-lactamases. The single amino acid substitutions P104K and K234R in KPC-2 affect the inactivation mechanism.

BACKGROUND

Prevalence of carbapenem resistant *Klebsiella pneumoniae*[1]

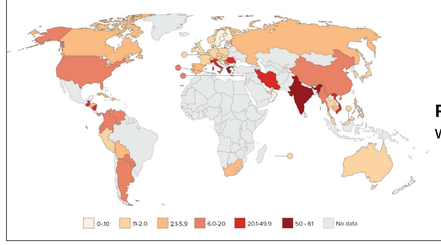

Figure 1: *K. pneumoniae* is a world-wide concern.

K. pneumoniae is one of the most common carbapenem-resistant *Enterobacteriaceae* (CRE)[2]

Figure 2: Centers for Disease Control and Prevention has declared carbapenem-resistant *K. pneumoniae* an urgent threat.

Structure of avibactam (Avi) and nacubactam (Nacu)[3]

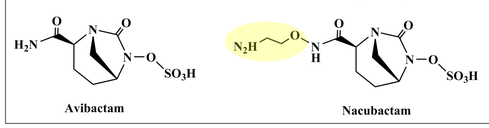

Figure 3: Diazabicyclooctane (DBO) ß-lactamase inhibitors.

Hypothesis: The meropenem-nacubactam combination will overcome antibiotic resistance in *Klebsiella pneumoniae* and inhibit KPC ß-lactamases.

METHODS & RESULTS

Nacubactam lowers the meropenem MICs in 44 clinical *Klebsiella pneumoniae* isolates.

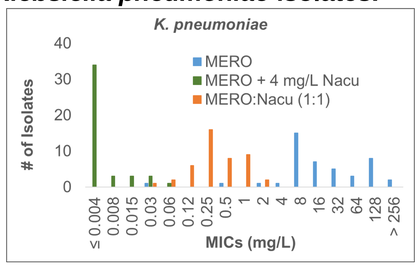

Table 1

	Nacu	Meropenem	Meropenem/Nacu (1:1 Ratio)	Meropenem/Nacu (Nacu 4 mg/L)
MIC_{50} (mg/L)	1	16	0.25	≤ 0.004
MIC_{90} (mg/L)	4	128	1	0.06

Figure 4: Microbroth dilution minimum inhibitory concentrations (MICs) of clinical *K. pneumoniae* strains conducted according to Clinical and Laboratory Standards Institute (CLSI)[3]. Meropenem R≥4.

K234R and P104K amino acid substitutions in KPC-2 led to slightly increased MICs to the meropenem-nacubactam without corresponding increases in meropenem MICs, compared to wild-type KPC-2.

Table 2

MICs (mg/L) of class A KPC-2 β-lactamases, expressed in *E. coli* DH10B

KPC-2 Substitution	Nacubactam	Meropenem/Nacubactam (4 mg/L Nacubactam)	Meropenem/Nacubactam (1:1 Ratio)	Meropenem
pBC SK vector				
wt	1	≤ 0.004	0.12	1
K73A	2	≤ 0.004	0.02	0.015
K73R	2	≤ 0.004	0.03	0.015
P104A	2	≤ 0.004	0.06	0.5
P104K	2	0.008	0.25	1
W105A	1	≤ 0.004	0.06	0.06
S130G	1	≤ 0.004	0.03	0.015
S130T	2	≤ 0.004	0.06	0.03
E166A	2	≤ 0.004	0.03	0.06
E166Y	2	≤ 0.004	0.03	0.03
N170A	4	0.008	0.06	0.03
N170P	2	≤ 0.004	0.06	0.03
R220K	1	≤ 0.004	0.06	0.06
R220M	1	≤ 0.004	0.03	0.03
T235A	2	≤ 0.004	0.03	0.03
T235S	2	≤ 0.004	0.03	0.12
T237A	1	≤ 0.004	0.03	0.06
T237S	1	≤ 0.004	0.06	0.5
V240G	2	≤ 0.004	0.25	4
V240K	2	0.015	0.25	2
E276A	1	≤ 0.004	0.12	0.5
E276N	2	≤ 0.004	0.06	0.25

Microbroth dilution MICs were performed on isogenic strains expressing single KPC-2 variants involved in ß–lactamase inhibition.

Table 3

MICs (mg/L) of class A KPC-2 β-lactamases, expressed in *E. coli* DH10B

KPC-2 Substitution	Nacubactam	Meropenem/Nacubactam (4 mg/L Nacubactam)	Meropenem/Nacubactam (1:1 Ratio)	Meropenem
pBR322 vector				
wt	2	≤ 0.004	0.25	8
R164A	2	≤ 0.004	0.25	0.5
R164H	2	≤ 0.004	0.25	2
R164P	2	≤ 0.004	0.06	0.03
R164S	2	≤ 0.004	0.12	1
D179A	2	≤ 0.004	0.06	0.03
D179C	1	≤ 0.004	0.06	0.06
D179E	1	≤ 0.004	0.03	0.015
D179F	2	≤ 0.004	0.06	0.06
D179G	4	0.008	0.12	0.12
D179H	2	≤ 0.004	0.06	0.03
D179I	1	≤ 0.004	0.02	0.015
D179K	2	≤ 0.004	0.03	0.03
D179L	2	≤ 0.004	0.06	0.03
D179M	2	≤ 0.004	0.06	0.06
D179N	2	≤ 0.004	0.25	2
D179P	1	≤ 0.004	0.03	0.03
D179Q	2	≤ 0.004	0.06	0.03
D179R	1	≤ 0.004	0.06	0.03
D179S	1	≤ 0.004	0.03	0.015
D179T	1	≤ 0.004	0.06	0.03
D179V	1	≤ 0.004	0.06	0.03
D179W	2	≤ 0.004	0.06	0.06
D179Y	2	≤ 0.004	0.06	0.06
R220A	2	≤ 0.004	0.12	0.12
K234A	1	≤ 0.004	0.02	0.015
K234R	2	≤ 0.004	0.5	2

Nacubactam and avibactam are more effective against KPC-2 wild-type than the K234R variant.

Table 4

E. coli DH10B	Nacubactam IC_{50} (µM)
pBR322-KPC-2	46
pBR322-K234R	781

Inhibition kinetics using *E. coli* periplasmic extract containing the pBR322 plasmid with KPC-2 or the K234R variant (above) and the purified ß-lactamases (right). Turnover was measured at a 1:1 ratio of ß-lactamase and DBO β-lactamase inhibitor after 24 hours of incubation.

Table 5

KPC-2	$K_{i\,app}$ (µM)	k_2/K ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	k_{cat}/k_{inact}
Nacubactam	31±3	5,815±582	0.0002	1
Avibactam[5,6]	1.0±0.1	21,580±2,100	0.00014	1

Table 6

K234R	$K_{i\,app}$ (µM)	k_2/K ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	k_{cat}/k_{inact}
Nacubactam	270±0.3	247 ±25	ND*	1
Avibactam	157±0.1	617±62	ND*	1

*not determinable.

Nacubactam and avibactam form stable adducts to KPC-2 and K234R β-lactamases.

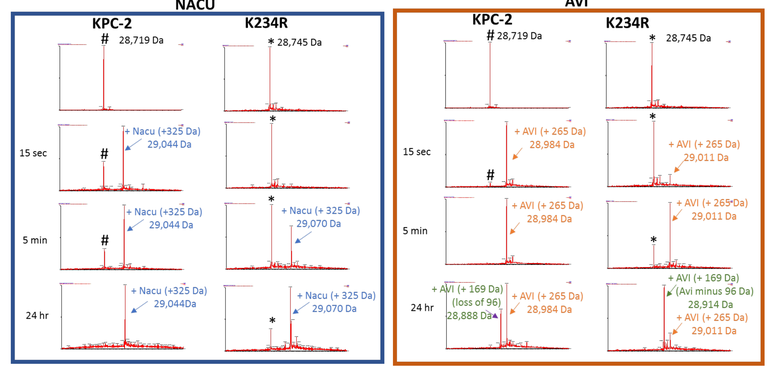

Figure 5: Electrospray-ionization mass spectrometry of KPC-2 or K234R β-lactamases (5 µg) incubated with nacubactam or avibactam at a 1:1 molar ratio at room temperature. Apo-KPC-2 (#) and Apo-K234R (*) are unbound. Avibactam is detected bound to ß-lactamase as an intact compound (265 Da) and de-sulfated (169 Da).

K234R is a disruptive substitution that leads to structural rearrangement of the active site

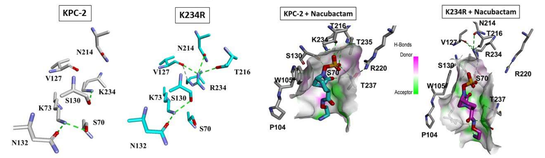

Figure 6: Molecular modeling shows that in the K234R variant, S130 and K73 are no longer poised for efficient proton shuttling during acylation.

CONCLUSIONS

- Nacubactam is a potent PBP DBO inhibitor partnered with meropenem against Enterobacteriaceae, including the strain expressing the clinically relevant and ceftazidime-avibactam resistant D179Y variant.
- The inherent antibacterial activity of nacubactam masks its inhibition of KPC-2 variant activity.
- The K234 residue in KPC-2 participates in the nacubactam inactivation mechanism, in part by shifting S130 and K73 into less optimal positions for acylation.
- ß-lactamase inhibition by DBOs is more efficient against KPC-2 than the K234R variant.
- The meropenem-nacubactam combination has potential to be an effective new antibiotic therapy against Enterobacteriaceae infections.

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