F-856

PDFs of this poster
may be downloaded at
www.rempexpharma.com

Pre-clinical Evaluation of a Carbapenem/ß-Lactamase Inhibitor Combination (RPX2003/RPX7009) Tested Against Serine-carbapenemase-producing Pathogens

M CASTANHEIRA, HK BECKER, PR RHOMBERG, RN JONES
JMI Laboratories, North Liberty, Iowa, USA

ICAAC 2012

JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
mariana-castanheira@jmilabs.com

Abstract

BACKGROUND: Serine-carbapenemase (SCase)-producing Enterobacteriaceae (ENT) is a matter of serious concern in the USA and several other countries. We evaluated the activity of a carbapenem/serine-ß-lactamase (BL) inhibitor (I) combination (Biapenem [BP; RPX2003]/RPX7009 [RPX9], here in BP/RPX9) tested against SCase-producing ENT.

METHODS: 167 SCase-producing ENT (160 KPC [± other BLs; 9 species], 6 SME and 1 NMC-A) were susceptibility (S) tested against RPX3/9 using checkerboard designed panels to assess the optimal BLI concentration (range, 0.5-32 µg/mL). BP and RPX9 were also tested alone.

RESULTS: Overall, RPX9 restored the activity of BP against SCase ENT isolates from 15.0% (RPX3 alone) to 95.8-98.8% of isolates inhibited at ≤2 µg/mL (Table). BP/RPX9 (MIC_{50}, ≤0.06 to 0.12 µg/mL for all BLI concentrations) was highly active against KPC-producing *K. pneumoniae* (34 strains), *E. coli* (15), *K. oxytoca* (10) and *R. planticola* (1). Against KPC-producers carrying 1-4 additional ß-lactamases (such as CTX-M, OXA, OXY and/or ESBL/narrow spectrum TEM and SHV), BP with RPX9 at 4, 8, 16 and 32 µg/mL inhibited 92.3, 96.7, 100.0 and 100.0% of the isolates at 1 µg/mL, respectively. KPC-producing ENT that hyperexpress chromosomal AmpC (*Enterobacter* spp. [25 strains], *C. freundii* [10] and *S. marcescens* [SM; 4]) had BP/RPX9 MIC_{50} values of ≤0.06 to 0.5 µg/mL (compared to BP alone MIC_{50}, 4 µg/mL). NMC-A-*E. cloacae* and SME-producing SM strains were all inhibited by ≤1 µg/mL RPX3 with RPX9 at 4, 8, 16 and 32 µg/mL. BLI showed no antibacterial activity when tested alone.

CONCLUSIONS: These results demonstrate that BP/RPX9 is a prime candidate for further development that could increase the treatment options against serious infections caused by SCase-producing pathogens.

Antimicrobial agent (167 strains tested)	No. of strains at MIC (µg/mL; cumulative %):								
	≤0.06	0.12	0.25	0.5	1	2	4	8	≥16
Biapenem	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.6)	6 (4.2)	18 (15.0)	29 (32.3)	31 (50.9)	82 (100.0)
+ RPX9 at 0.5 µg/mL	29 (17.4)	34 (37.7)	24 (52.1)	20 (64.1)	14 (72.5)	12 (79.6)	4 (82.0)	8 (86.8)	22 (100.0)
+ RPX9 at 1 µg/mL	54 (32.3)	33 (52.1)	21 (64.7)	8 (69.5)	18 (80.2)	4 (82.6)	6 (86.2)	7 (90.4)	16 (100.0)
+ RPX9 at 2 µg/mL	77 (46.1)	29 (63.5)	12 (70.7)	14 (79.0)	7 (83.2)	9 (88.6)	8 (93.4)	4 (95.8)	7 (100.0)
+ RPX9 at 4 µg/mL	85 (50.9)	23 (64.7)	21 (77.2)	11 (83.8)	13 (91.6)	7 (95.8)	4 (98.2)	1 (98.8)	2 (100.0)
+ RPX9 at 8 µg/mL	84 (50.3)	28 (67.1)	27 (83.2)	12 (90.4)	10 (96.4)	3 (98.2)	1 (98.8)	1 (99.4)	1 (100.0)
+ RPX9 at 16 µg/mL	83 (49.7)	33 (69.5)	18 (80.2)	20 (92.2)	11 (98.8)	0 (98.8)	0 (98.8)	1 (99.4)	1 (100.0)
+ RPX9 at 32 µg/mL	58 (34.7)	43 (60.5)	26 (76.0)	22 (89.2)	15 (98.2)	1 (98.8)	0 (98.8)	2 (100.0)	

Introduction

Bacterial isolates resistant to clinically available ß-lactams present a challenge to successful treatment of serious infections. ß-lactamase-mediated resistance, in particular, represents a significant clinical threat because of the mobile nature of the genes encoding these enzymes. Two strategies have been used to restore the utility of ß-lactam compounds: (i) the design/discovery of novel ß-lactam molecules that are refractory to enzymatic inactivation, and (ii) the inhibition of ß-lactamases, thereby allowing the ß-lactam to retain target concentrations.

Carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and -negative bacteria among hundreds of different ß-lactam compounds. These antimicrobial agents play a critically important role in the antimicrobial armamentarium due to greater stability to many ß-lactamases. However, several recent studies clearly show that resistance to carbapenems is increasing throughout the world and the recent spread of carbapenem-resistant pathogens, including those producing KPC enzymes, seriously threatens the use of the carbapenem class.

In this study, we evaluated the activity of a carbapenem (Biapenem) combined with a ß-lactamase inhibitor (RPX7009) tested against a collection of 167 Enterobacteriaceae strains producing serine-carbapenemases collected worldwide, including 160 KPC-producing strains.

Materials and Methods

BACTERIAL ISOLATES. A total of 167 clinical isolates of Enterobacteriaceae producing serine-carbapenemases were tested. Genes encoding serine-carbapenemases and other ß-lactamases were previously identified using various PCR strategies; and amplicons were sequenced on both strands. Isolates selected from multiple countries and years to provide genetic diversity and only one strain from clonal outbreaks was included. For analysis purposes, isolates were divided in four groups: (i) *Klebsiella* spp. (*Klebsiella pneumoniae* [34 strains] and *K. oxytoca* [10 strains]), *E. coli* (15 strains) and one *Raoultella planticola* (1 strain) producing KPC enzymes (60 strains; KPC-2 and KPC-3); (ii) KPC-producing Enterobacteriaceae carrying multiple ß-lactamases (61 strains; Table 1); (iii) Strains producing KPC enzymes that putatively hyperexpress chromosomal cephalosporinases (AmpC, 39 strains; KPC-2, KPC-3 and KPC-4; *Enterobacter* spp. [25 strains], *Citrobacter freundii* [10 strains], *Serratia marcescens* [4 strains]; (iv) Isolates producing non-KPC serine-carbapenemases (7 strains): one NMC-A-producing *E. cloacae* and six SME-producing *Serratia marcescens.*

SUSCEPTIBILITY TESTING. MIC values were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology as described in CLSI document M07-A9 (2012). The carbapenem biapenem (concentration range, 0.06-64 µg/mL) and the ß-lactamase inhibitor RPX7009 (concentration range 0.5-32 µg/mL) were tested in a checkerboard configuration using cation-adjusted Mueller-Hinton broth as previously described. Quality control (QC) ranges and interpretive criteria were those published in CLSI M100-S22 (2012); tested QC strains included *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and KPC-producing *K. pneumoniae* BAA 1705, and all QC results were within published ranges.

Results

- Overall, the activity of biapenem (MIC_{50}, 8 µg/mL) against 167 serine-carbapenemase-producing Enterobacteriaceae was enhanced by at least 32-fold in the presence of various concentrations (0.5 - 32 µg/mL) of the ß-lactamase inhibitor RPX7009 (Table 2).
- Susceptibility rates when applying current CLSI breakpoints for imipenem or meropenem (≤1 µg/mL as susceptible) were only 4.2% susceptible for biapenem alone and 72.5 to 98.8% when this carbapenem was tested with increasing concentrations of RPX7009 (Table 2).
- Inhibitor concentrations of 4 and 8 µg/mL increased biapenem potency by at least 128-fold (MIC_{50}, ≤0.06 µg/mL for both combinations; Tables 2 and 3). Susceptibility rates greater than 90.0% were observed for inhibitor concentrations of 4, 8, 16 and 32 µg/mL (91.6, 96.4, 98.8 and 98.2% susceptible, respectively; Table 2).
- Against KPC-producing *K. pneumoniae* (34 strains), *E. coli* (15), *K. oxytoca* (10) and *R. planticola* (1), biapenem/RPX7009 was highly active for all inhibitor concentrations tested ($MIC_{50/90}$, ≤0.06/0.5-1 µg/mL; Table 3). Susceptibility rates (≤1 µg/mL, CLSI breakpoint) were 93.3 and 96.7% when using 4 and 8 µg/mL of RPX7009 (Table 3).
- When biapenem was tested with a fixed concentration of 4 µg/mL of RPX7009, 88.5 and 95.1% of the KPC-producers were known to carry additional ß-lactamases (listed in Table 1) were inhibited at current CLSI and EUCAST breakpoints of ≤1 µg/mL, respectively (Table 3).
- Biapenem/RPX7009 combinations were all very active against KPC-producing strains from bacterial species that putatively hyperproduce chromosomal AmpC enzymes (MIC_{50}, 0.12 and ≤0.06 µg/mL using 4 and 8 µg/mL of the inhibitor; Table 3).
- NMC-A-producing *E. cloacae* and SME-producing *S. marcescens* were all inhibited by ≤1 µg/mL when RPX7009 concentrations of 4 and 8 µg/mL were tested with biapenem (Table 3).
- When tested alone, the BLI displayed no activity against these serine-carbapenemase-producing Enterobacteriaceae strains (all MIC values at >32 µg/mL; Table 2).

TABLE 1. β-lactamase Types Detected on 61 KPC-producing Enterobacteriaceae Strains Carrying Other β-lactamase Encoding Genes (from USA Isolates Recovered from 2007 and 2010).

Organism (no. tested)	ß-lactamase detected
K. pneumoniae (4)	CTX-M-15-like + KPC-2 + SHV-1 + TEM-1
K. pneumoniae (1)	CTX-M-15-like + KPC-3 + OXA-1/30 + SHV-11 + TEM-1
R. planticola (1)	FOX-5 + KPC-3 + PSE-1 + SHV-7
K. oxytoca (1)	KPC-2 + OXA-2-like + OXY-like
K. oxytoca (1)	KPC-2 + OXA-2-like + SHV-30
K. oxytoca (1)	KPC-2 + OXY-1 + SHV-40
K. pneumoniae (1)	KPC-2 + SHV-1
K. pneumoniae (2)	KPC-2 + SHV-1 + TEM-1
K. pneumoniae (1)	KPC-2 + SHV-11
K. pneumoniae (10)	KPC-2 + SHV-11 + TEM-1
K. pneumoniae (6)	KPC-2 + SHV-12 + TEM-1
K. pneumoniae (1)	KPC-2 + SHV-27 + TEM-1
K. pneumoniae (1)	KPC-2 + SHV-30 + TEM-1
E. cloacae (2)	KPC-2 + TEM-1
K. pneumoniae (2)	KPC-2 + TEM-1
K. pneumoniae (3)	KPC-3 + SHV-1 + TEM-1
K. pneumoniae (11)	KPC-3 + SHV-11 + TEM-1
E. cloacae (2)	KPC-3 + SHV-12 + TEM-1
K. pneumoniae (2)	KPC-3 + SHV-12 + TEM-1
R. ornithinolytica (1)	KPC-3 + SHV-7 + TEM-1
K. pneumoniae (2)	KPC-3 + SHV-like + TEM-1
E. cloacae (2)	KPC-3 + TEM-1
K. pneumoniae (3)	KPC-3 + TEM-1

TABLE 2. MIC Distribution for Biapenem Alone and in Combination with RPX7009 When Tested Against 167 Serine-carbapenemase-producing Enterobacteriaceae Strains.

Antimicrobial agent (167 strains tested)	No. of strains at MIC (µg/mL; cumulative %):[a]												MIC (µg/mL)		% Susc.
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	> 64	50%	90%	CLSI[b]/EUCAST[c]
Biapenem	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.6)	6 (4.2)	18 (15.0)	29 (32.3)	31 (50.9)	44 (77.2)	8 (82.0)	17 (92.2)	13 (100.0)	8	64	4.2/15.0
+ RPX7009 at 0.5 µg/mL	29 (17.4)	34 (37.7)	24 (52.1)	20 (64.1)	14 (72.5)	12 (79.6)	4 (82.0)	8 (86.8)	5 (89.8)	9 (95.2)	5 (98.2)	3 (100.0)	0.25	32	72.5/79.6
+ RPX7009 at 1 µg/mL	54 (32.3)	33 (52.1)	21 (64.7)	8 (69.5)	18 (80.2)	4 (82.6)	6 (86.2)	7 (90.4)	7 (94.6)	5 (97.6)	3 (99.4)	1 (100.0)	0.12	8	80.2/82.6
+ RPX7009 at 2 µg/mL	77 (46.1)	29 (63.5)	12 (70.7)	14 (79.0)	7 (83.2)	9 (88.6)	8 (93.4)	4 (95.8)	5 (98.8)	1 (99.4)	0 (99.4)	1 (100.0)	0.12	4	83.2/88.6
+ RPX7009 at 4 µg/mL	85 (50.9)	23 (64.7)	21 (77.2)	11 (83.8)	13 (91.6)	7 (95.8)	4 (98.2)	1 (98.8)	1 (99.4)	0 (99.4)	0 (99.4)	1 (100.0)	≤0.06	1	91.6/95.8
+ RPX7009 at 8 µg/mL	84 (50.3)	28 (67.1)	27 (83.2)	12 (90.4)	10 (96.4)	3 (98.2)	1 (98.8)	1 (99.4)	0 (99.4)	0 (99.4)	1 (100.0)		≤0.06	0.5	96.4/98.2
+ RPX7009 at 16 µg/mL	83 (49.7)	33 (69.5)	18 (80.2)	20 (92.2)	11 (98.8)	0 (98.8)	0 (98.8)	1 (99.4)	1 (100.0)				0.12	0.5	98.8/98.8
+ RPX7009 at 32 µg/mL	58 (34.7)	43 (60.5)	26 (76.0)	22 (89.2)	15 (98.2)	1 (98.8)	0 (98.8)	2 (100.0)					0.12	1	98.2/98.8
RPX7009 alone	-	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	167 (100.0)[d]	-	-	>32	>32	NA[e]

a. - = untested MIC.
b. The CLSI breakpoint criteria (M100-S22; 2012) for imipenem/meropenem was applied for comparison purposes.
c. The EUCAST breakpoint criteria (2012) for imipenem/meropenem was applied for comparison purposes.
d. Isolates were inhibited by >32 µg/mL of RPX7009.
e. NA = not applicable.

TABLE 3. MIC Distribution for Biapenem in Combination with RPX7009 at 4 and 8 µg/ml When Tested Against Groups of Carbapenemase-producing Strains.

Organism group (no. tested)	No. of strains at biapenem/RPX7009 MIC (µg/mL; cumulative %):									MIC (µg/mL)		% Susc.
	≤0.06	0.12	0.25	0.5	1	2	4	8	≥16	50%	90%	CLSI[a]/EUCAST[b]
KPC-producers (60)												
RPX7009 at 4 µg/mL	38 (63.3)	6 (73.3)	1 (75.0)	4 (81.7)	7 (93.3)	2 (96.7)	0 (96.7)	1 (98.3)	1 (100.0)	≤0.06	1	93.3/96.7
RPX7009 at 4 µg/mL	36 (60.0)	8 (73.3)	6 (83.3)	5 (91.7)	3 (96.7)	0 (96.7)	0 (96.7)	1 (98.3)	1 (100.0)	≤0.06	0.5	96.7/96.7
KPC-producers carrying other ß-lactamase encoding genes (61)												
RPX7009 at 4 µg/mL	28 (45.9)	9 (60.7)	10 (77.0)	3 (82.0)	4 (88.5)	4 (95.1)	3 (100.0)			0.12	2	88.5/95.1
RPX7009 at 8 µg/mL	27 (44.3)	10 (60.7)	14 (83.6)	4 (90.2)	4 (96.7)	2 (100.0)				0.12	0.5	96.7/100.0
KPC-producers from species that hyperproduce chromosomal AmpC (39)												
RPX7009 at 4 µg/mL	18 (46.2)	8 (66.7)	7 (84.6)	1 (87.2)	2 (92.3)	1 (94.9)	1 (97.4)	0 (97.4)	1 (100.0)	0.12	1	92.3/94.9
RPX7009r at 8 µg/mL	20 (51.3)	10 (76.9)	4 (87.2)	0 (87.2)	3 (94.9)	1 (97.4)	1 (100.0)			≤0.06	1	94.9/97.4
SME- or NMC-A-producers (7)												
RPX7009 at 4 µg/mL	1 (14.3)	0 (14.3)	3 (57.1)	3 (100.0)						0.25	-	100.0/100.0
RPX7009 at 8 µg/mL	1 (14.3)	0 (14.3)	3 (57.1)	3 (100.0)						0.25	-	100.0/100.0

a. The CLSI breakpoint criteria (M100-S22; 2012) for imipenem/meropenem was applied for comparison purposes.
b. The EUCAST breakpoint criteria (2012) for imipenem/meropenem was applied for comparison purposes.

Conclusions

- The ß-lactamase inhibitor RPX7009 at concentrations of 4 µg/mL or greater consistently restored the activity of the carbapenem biapenem when tested against Enterobacteriaceae strains that produce serine-carbapenemases alone or in combination with other non-Ambler class B ß-lactamases.
- The combination of Biapenem/RPX7009 showed excellent activity against these troublesome serine-carbapenemase-producing strains. These organisms are often multidrug-resistant, carry additional ß-lactamases (including ESBLs) and therapeutic options to treat infections caused by these pathogens are limited.

ACKNOWLEDGEMENTS

This study was sponsored by a research grant from Rempex Pharmaceuticals Inc.

REFERENCES

1. Bale MJ, Jones RN, Erwin ME (1994). Minimum inhibitory concentration quality-control guidelines for biapenem, DU-6859a, FK-037, levofloxacin, grepafloxacin, and ceftizoxime when using various National Committee for Clinical Laboratory Standards susceptibility test methods. Quality Control Study Group. *Diagn Microbiol Infect Dis* 19: 65-68.
2. Castanheira M, Mendes RE, Woosley LN, Jones RN (2011). Trends in carbapenemase-producing *Escherichia coli* and *Klebsiella* spp. from Europe and the Americas: Report from the SENTRY Antimicrobial Surveillance Programme (2007-09). *J Antimicrob Chemother* 66: 1409-1411.
3. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition.* Wayne, PA: CLSI.
4. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement.* Wayne, PA: CLSI.
5. Drawz SM, Bonomo RA (2010). Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* 23: 160-201.
6. European Committee on Antimicrobial Susceptibility Testing (2012). Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0, January 2012. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed January 1, 2012.
7. Hoban DJ, Jones RN, Yamane N, Frei R, Trilla A, Pignatari AC (1993). In vitro activity of three carbapenem antibiotics. Comparative studies with biapenem (L-627), imipenem, and meropenem against aerobic pathogens isolated worldwide. *Diagn Microbiol Infect Dis* 17: 299-305.
8. Moody J (2004). *Susceptibility tests to evaluate synergism.* In *Clinical Microbiology Procedures Handbook*. Washington, D.C.: ASM Press. 5.12.11-5.12.23.
9. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA (2011). Carbapenems: past, present, and future. *Antimicrob Agents Chemother* 55: 4943-4960.
10. Pereira M, Perilli M, Mantengoli E, Luzzaro F, Toniolo A, Rossolini GM, Amicosante G (2000). PER-1 extended-spectrum beta-lactamase production in an *Alcaligenes faecalis* clinical isolate resistant to expanded-spectrum cephalosporins and monobactams from a hospital in Northern Italy. *Microb Drug Resist* 6: 85-90.
11. Perry CM, Ibbotson T (2002). Biapenem. *Drugs* 62: 2221-2234.