B-078

Efficacy of Carbavance (Meropenem+RPX7009) against Carbapenem-resistant *E. coli* and *K. pneumoniae* in a Murine UTI Model

55th ICAAC / 58th ICC
San Diego, CA
September 17-21, 2015

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Abstract

Background: Resistance in Enterobacteriaceae due to the increasing variety of β-lactamase enzymes, particularly carbapenemases, has become an issue of clinical importance. RPX7009 is a member of a new class of beta-lactamase inhibitors with inhibitory activity against important serine carbapenemases, including KPC, and is being developed in combination with meropenem. The objective of these studies was to demonstrate the efficacy of meropenem (MER) in combination with RPX7009 (RPX) in a murine urinary tract infection model.
Methods: Female C3H/HeJ mice were placed on 5% glucose water 6 days pre- infection. Animals were transurethrally infected with 1.52 x 10e9 CFU *E. coli* UNT167-1 (meropenem MIC 32 mg/L; meropenem + RPX7009 MIC ≤0.06 mg/L). Treatments were initiated 4 days post-infection and continued every 2 hours for 24 hours. Untreated controls were sacrificed at the start of treatment and treated groups were sacrificed 24 hours after the start of treatment, kidneys were removed, homogenized, and plated to determine colony counts
Results: MER/RPX7009 produced 1.3 to 2.0 logs greater bacterial killing in kidneys compared to MER alone.

E. coli UNT167-1 (KPC-2, SHV-12 beta-lactamases)	Dose (mg/kg/Dose)	Change in Log CFU/pair kidneys
Untreated	NA	0.47
Meropenem	100	-0.84
Meropenem	300	-0.82
Meropenem/RPX7009	100 + 25	-2.21
Meropenem/RPX7009	100 + 50	-2.51
Meropenem/RPX7009	300 + 50	-2.8

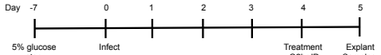
Conclusion: Carbavance produced significant bacterial killing in kidneys infected with a meropenem-resistant strain of *E. coli*. These data suggest that this combination may have utility in the treatment of complicated urinary tract infections due to KPC-producing, carbapenem-resistant Enterobacteriaceae.

Introduction

Bacterial isolates, resistant to clinically available β-lactams, present a challenge to successful treatment of serious infections. β-lactamase mediated resistance, in particular, represents a significant clinical threat because of the mobile nature of the genes encoding these enzymes. Carbapenems possess the broadest spectrum of activity and play a critical role in treatment due to greater stability to many β-lactamases. Studies show that resistance to carbapenems is increasing worldwide and the recent spread of carbapenem-resistant pathogens, including those producing KPC (*K. pneumoniae* carbapenemase) enzymes seriously threatens the use of the carbapenem class. β-lactamase inhibitors have been successfully used to restore/maintain the efficacy of several β-lactams, and in this study, we evaluated the activity of meropenem combined with RPX7009, a new β-lactamase inhibitor against *E. coli* and *K. pneumoniae* strains expressing the KPC enzyme in a murine model of ascending urinary tract infection.

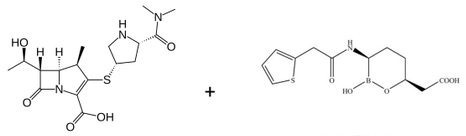
Methods and Materials

Minimum inhibitory concentrations (MICs): MICs were determined for meropenem w/wo RPX7009 against the test strains by using the microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI).
Strains: *Escherichia coli* UNT167-1 (KPC-2 + SHV-12) and *Klebsiella pneumoniae* UNT170-1 (KPC-2) clinical isolates.
Animals: Female C3H/Hej mice (22 ± 2 g).
Preparation: Mice were kept on water with 5% glucose starting 7 days prior to infection and for the duration of the study.
Infection: Anesthetized mice were trans-urethrally injected with 40 - 50 uL of a prepared inoculum (8 – 9 Log10 CFU) via a PE10 catheter. The bacteria ascend the urinary tract and localize in the kidneys by 4 days after infection.



Treatment: Meropenem alone or co-administered with RPX7009 via intraperitoneal (IP) injection, every two hours (q2hr) starting 4 days after infection.
Sampling / Endpoint: Kidneys were collected in sterile PBS, homogenized, diluted and plated for CFU enumeration at the start of treatment and 2 hours after the last administered dose.

Meropenem + RPX7009



Meropenem
1 β-methyl carbapenem

RPX7009
Cyclic boronic acid
β-lactamase inhibitor

Minimum Inhibitory Concentrations (MIC)

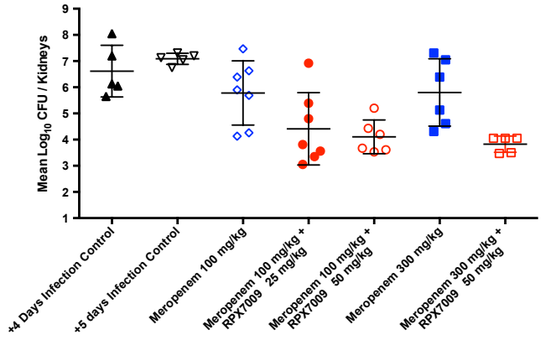
Strain	Organism	Phenotype	MIC (ug/mL) Meropenem	MIC (ug/mL) Meropenem + RPX7009*
UNT167-1	*Escherichia coli*	KPC-2 + SHV-12	32	≤0.06
UNT170-1	*Klebsiella pneumoniae*	KPC-2	32	≤0.06

* RPX7009 at a constant 8 μg/mL

- *E. coli* and *K. pneumoniae* clinical isolates expressing the KPC-2 β-lactamase were resistant to Meropenem.
- The addition of the β-lactamase inhibitor RPX-7009 decreased meropenem MICs by >256-fold thereby rendering both strains susceptible to the carbapenem.

Efficacy of Meropenem alone or in combination with RPX7009 against an *E. coli* UNT167-1 UTI Infection

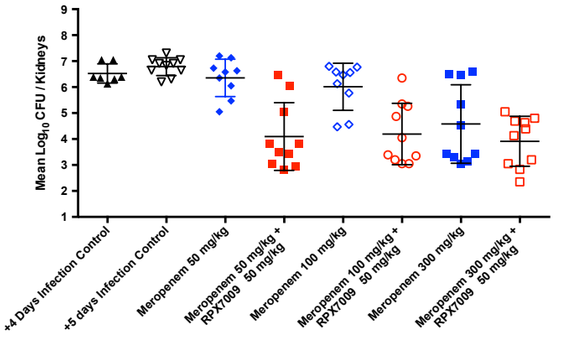
Test Article	mg/kg/dose	Mean Log_{10} CFU Kidneys	Mean Log_{10} CFU SD	Change in Log_{10} CFU vs Day 4	Change in Log_{10} CFU vs Mero alone
Meropenem	100	5.78	1.23	-0.84	na
	300	5.80	1.29	-0.82	na
Meropenem + RPX7009	100 + 25	4.41	1.38	-2.20	-1.37
	100 + 50	4.11	0.64	-2.51	-1.67
	300 + 50	3.82	0.31	-2.79	-1.98
Infection Controls	Day 4	6.62	0.98	na	na
	Day 5	7.09	0.21	0.47	na



- Transurethral inoculation of *E. coli* UNT167-1 established an ascending UTI in mice with 6.62 – 7.09 mean log10 CFU bacterial titers in the kidneys at 4 to 5 days post-infection.
- When compared to 4 day untreated controls, meropenem alone at doses of 100 and 300 mg/kg q2hr reduced the bacterial load in the kidneys by < 1 log10 CFU.
- The combination of Meropenem + RPX7009 resulted in bacterial kidney titers that were 2.20 – 2.79 log10 CFU lower than the untreated control groups on Days 4 and 5.
- Meropenem efficacy at doses of 100 and 300 mg/kg was enhanced with the addition of RPX7009 resulting in 1.37 – 1.98 log10 CFU lower kidney titers compared to Meropenem alone at the same doses.

Efficacy of Meropenem alone or in combination with RPX7009 against a *K. pneumoniae* UNT170-1 UTI Infection

Test Article	mg/kg/dose	Mean Log_{10} CFU Kidneys	Mean Log_{10} CFU SD	Change in Log_{10} CFU vs Day 4	Change in Log_{10} CFU vs Mero alone
Meropenem	50	6.35	0.72	-0.18	na
	100	6.01	0.91	-0.52	na
	300	4.58	1.50	-1.95	na
Meropenem + RPX7009	50 + 50	4.10	1.30	-2.43	-2.25
	100 + 50	4.19	1.18	-2.34	-1.82
	300 + 50	3.91	0.96	-2.62	-0.67
Infection Controls	Day 4	6.53	0.37	na	na
	Day 5	6.79	0.34	0.26	na



- Transurethral inoculation of *K. pneumoniae* UNT170-1 established a chronic infection in mice with 6.53 – 6.79 mean log10 CFU bacterial titers in the kidneys at 4 to 5 days post-infection.
- Meropenem alone at doses of 50 and 100 mg/kg q2hr exhibited a minimal effect on the bacterial load in the kidneys.
- The combination of Meropenem + RPX7009 resulted in bacterial kidney titers that were 2.34 – 2.62 log10 CFU lower than the untreated control groups on Days 4 and 5.
- The efficacy of Meropenem at doses of 50 and 100 mg/kg q2hr was enhanced with the addition of RPX7009 resulting in a 1.82 – 2.25 log10 CFU reduction in the kidneys when compared to Meropenem alone at the same doses.

Summary and Conclusions

- Although carbapenems are widely recognized as an effective class of antimicrobials, carbapenem-resistant Enterobacteriaceae, due to the carbapenem hydrolyzing β-lactamase KPC, now threaten the usefulness of all β-lactam antibiotics for the treatment of serious infections.
- In the presence of RPX7009, a novel β-lactamase inhibitor, two KPC producing / meropenem-resistant clinical isolates (*E. coli and K. pneumoniae*) were rendered susceptible to meropenem (MIC ≤ 0.06 μg/mL).
- In the mouse urinary tract infection model, meropenem alone was mostly ineffective at reducing the bacterial burden of either isolate in the kidneys of infected animals.
- The addition of RPX7009 to the Meropenem regimen greatly enhanced the effectiveness of Meropenem in reducing the bacterial infection in the kidneys by a clinically meaningful extent.
- The results indicate that Meropenem + RPX7009 may be an effective therapeutic option for the treatment of urinary tract infections caused by carbapenem-resistant Enterobacteriaceae. Clinical studies are in progress.

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Acknowledgments

The mouse urinary tract infection model for ascending pyelonephritis was conducted in accordance with the protocols approved by the UNTHSC-Institutional Animal Care and Use Committee. This project has been funded in whole or in part with federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority (BARDA), under Contract No.HHSO100201400002 with Rempex Pharmaceuticals, a wholly-owned subsidiary of The Medicines Company