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PHARMACOLOGY

Activity of Meropenem-Vaborbactam against Carbapenem-Resistant *Enterobacteriaceae* in a Murine Model of Pyelonephritis

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ABSTRACT The recently approved combination of meropenem and vaborbactam (Vabomere) is highly active against Gram-negative pathogens, especially *Klebsiella pneumoniae* carbapenemase (KPC)-producing, carbapenem-resistant *Enterobacteriaceae*. We evaluated the efficacy of meropenem-vaborbactam against three clinically relevant isolates in a murine pyelonephritis model. The data indicate that the combination of meropenem and vaborbactam significantly increased bacterial killing compared to that with the untreated controls. These data suggest that this combination may have utility in the treatment of complicated urinary tract infections due to KPC-producing, carbapenem-resistant *Enterobacteriaceae*.

KEYWORDS vaborbactam, meropenem, pyelonephritis, *Klebsiella pneumoniae* carbapenemase, KPC

Bacterial isolates that are resistant to clinically available beta-lactams, including carbapenems, present a growing challenge to the successful treatment of these serious infections. Carbapenems possess the broadest spectrum of activity and play a critical role in the treatment of multidrug-resistant bacterial infections, but studies have shown that resistance to carbapenems is increasing worldwide (1), seriously threatening the use of the carbapenem class. In fact, carbapenem-resistant *Klebsiella* spp. and *Escherichia coli* cause an estimated 9,300 health care-associated infections each year in the United States (2).

Beta-lactamase inhibitors have been successfully used to restore or maintain the efficacy of several beta-lactams. One such beta-lactamase inhibitor is the recently discovered vaborbactam, which is a novel cyclic boronic acid-based beta-lactamase inhibitor that restores the activity of beta-lactams against *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria (3). The beta-lactam–beta-lactamase combination of meropenem and vaborbactam has demonstrated both *in vitro* and *in vivo* activities against KPC-producing strains of *Enterobacteriaceae* (4–6).

In this study, we evaluated the activity of meropenem combined with vaborbactam against *E. coli* and *K. pneumoniae* strains expressing the KPC enzyme in a murine model of pyelonephritis.

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For both susceptibility and efficacy studies, meropenem for injection (lot no. DF-3297; Sandoz) was purchased from commercial sources. Vaborbactam (lot no. P-232-159-2) was manufactured by The Medicines Company. Meropenem was dissolved according to the package insert. Vaborbactam was solubilized in water and the pH adjusted by the addition of NaOH.

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TABLE 1 MICs of meropenem alone and in combination with 8 μg/ml vaborbactam for the *Enterobacteriaceae* strains used in the study

Strain	Organism	Source (type [location])	Phenotype	MIC (μg/ml)	
				Meropenem	Meropenem plus vaborbactam
UNT170-1	*K. pneumoniae*	Urine (Puerto Rico)	KPC-2	32	≤0.06
UNT171-1	*K. pneumoniae*	Urine (Greece)	KPC-2	32	≤0.06
UNT167-1	*E. coli*	Gallbladder (USA)	KPC-2, SHV-12	32	≤0.06

Prior to efficacy studies, the susceptibility to meropenem alone or in combination with vaborbactam was determined by a broth microdilution assay according to CLSI reference methods (7). As shown in Table 1, the 3 strains used in this study (*K. pneumoniae* UNT170-1 and UNT171-1 and *E. coli* UNT167-1), all clinical isolates expressing the KPC-2 beta-lactamase, were resistant to meropenem, with MICs of 32 μg/ml. The addition of the beta-lactamase inhibitor vaborbactam decreased meropenem MICs by >256-fold to ≤0.06 μg/ml, thereby rendering all 3 strains susceptible to meropenem.

We have previously shown the efficacy of the meropenem-vaborbactam combination in a thigh infection model (6). Here, we examined the efficacy of meropenem alone or in combination with vaborbactam in a pyelonephritis model against three KPC-producing strains, with 2 *K. pneumoniae* strains and one *Escherichia coli* strain. In these studies, female C3H/HeJ mice (22 ± 2 g) used were obtained from Jackson Laboratories. Animals were provided food and water *ad libitum* in accordance with National Institutes of Health guidelines for the care and use of laboratory animals (8). Mice were maintained on 5% glucose water starting 6 days prior to infection and for the duration of the study. This regimen led to diuresis, which did not have ill effects on the animals but did enhance the ability of the bacteria utilized in the study to infect their hosts. Mice were anesthetized with ketamine HCl (40 mg/kg of body weight) and xylazine (6 mg/kg of body weight) in 0.15 ml of phosphate-buffered saline (PBS) injected intraperitoneally. Using a dissecting scope with ×10 magnification, the urethral orifice was located. A tapered PE10 catheter from Braintree Scientific (Braintree, MA) was inserted, and 50 μl of inoculum was slowly injected. The bacteria then ascended through the urinary tract and localized in the kidney, establishing a site of infection. Untreated controls were euthanized at the start of treatment to determine baseline bacterial counts. All treated animals were euthanized 2 h after the last administered dose. At this time, both kidneys were collected in sterile PBS, homogenized, serially diluted, and plated on Mueller-Hinton agar for the determination of bacterial counts. Bacterial counts in kidneys were analyzed using the unpaired *t* test (GraphPad Prism, version 6.03). A *P* value of <0.05 was considered statistically significant.

Treatment regimens were chosen in order to simulate exposures in humans. Briefly, meropenem administered at 100 or 300 mg/kg every 2 h over 24 h in mice produces an exposure equivalent to 1 or 2 g of meropenem administered every 8 h by 3-h infusion in humans, respectively (9). Vaborbactam administered at 25 or 50 mg/kg every 2 h for 24 h in mice produces an exposure equivalent to 1 or 2 g of vaborbactam administered every 8 h by 3-h infusion in humans, respectively (9). All doses were administered intraperitoneally every 2 h, for a total of 12 doses.

The results of the murine pyelonephritis model with *K. pneumoniae* UNT170-1 are presented in Table 2. A total of 16 animals were infected, treated, and evaluated against a total of 10 control animals that were infected but not treated. Transurethral inoculation of the *K. pneumoniae* UNT170-1 isolate established pyelonephritis in the study animals, with mean titers of 6.39 and 6.74 $\log_{10}$ CFU in the kidneys at 4 (start of treatment) and 5 days postinfection, respectively. Compared to the day 4 untreated controls, meropenem alone administered intraperitoneally at a dose of 300 mg/kg every 2 h reduced the bacterial load in the kidneys by an average of 1.51 $\log_{10}$ CFU ($P = 0.0015$). The combination of meropenem and vaborbactam at a dose of 300 mg/kg

TABLE 2 Efficacy of meropenem alone and in combination with vaborbactam against *K. pneumoniae* UNT170-1 in a murine pyelonephritis model[a]

Test	Dose (mg/kg) or day	*n*	Kidney $\log_{10}$ CFU		Change in $\log_{10}$ CFU vs:		*P*
			Mean	SD	Day 4	Meropenem alone	
Meropenem	300	8	4.88	0.55	−1.51	NA	0.0015 vs untreated day 4; 0.00035 vs 300 + 50 mg/kg
Meropenem + vaborbactam	300 + 50	8	3.5	0.94	−2.89	−1.38	0.0017 vs untreated day 4
Untreated controls	Day 4	2	6.39	0.37	NA	NA	NA
	Day 5	8	6.74	0.64	0.35	NA	0.246 vs untreated day 4

[a]NA, not applicable.

plus 50 mg/kg given every 2 h for 24 h resulted in bacterial kidney titers that were 2.89 $\log_{10}$ CFU lower than the day 4 controls ($P = 0.0017$) and 1.38 $\log_{10}$ CFU lower than meropenem alone ($P = 0.0015$) at the same administered dose.

The results of the murine pyelonephritis model with *K. pneumoniae* UNT171-1 are shown in Table 3. A total of 33 animals were infected, treated, and evaluated against control animals that were infected but left untreated. Transurethral inoculation of the *K. pneumoniae* UNT171-1 isolate established pyelonephritis in mice, with mean bacterial counts of 3.73 and 4.05 $\log_{10}$ CFU in the kidneys at 4 and 5 days postinfection, respectively. Meropenem alone at a dose of 100 mg/kg every 2 h exhibited a minimal effect on the bacterial load in the kidneys, while a higher dose of 300 mg/kg led to a 1.38-$\log_{10}$ CFU reduction in bacterial kidney titers compared to the untreated control day 4 titers ($P = 0.021$). The combination of meropenem at the same doses with vaborbactam at a dose of either 50 or 25 mg/kg resulted in bacterial kidney titers that were 1.25 ($P = 0.021$) to 1.33 ($P = 0.024$) $\log_{10}$ CFU lower than those of the untreated control groups on day 4. Meropenem efficacy at 100 mg/kg was enhanced with the addition of vaborbactam, resulting in 1.42 to 1.48 $\log_{10}$ CFU lower kidney titers than those with meropenem alone at the same dose ($P < 0.05$).

The efficacy of meropenem alone and in combination with vaborbactam against the *E. coli* UNT167-1 isolate is presented in Table 4. Transurethral inoculation with the *E. coli* UNT167-1 isolate established pyelonephritis in mice with mean bacterial titers of 6.62 and 7.09 $\log_{10}$ CFU in the kidneys at 4 and 5 days postinfection, respectively. Compared to the day 4 untreated controls, meropenem alone at doses of 100 and 300 mg/kg every 2 h reduced the bacterial load in the kidneys by <1 $\log_{10}$ CFU ($P < 0.05$). Meropenem at the same doses in combination with vaborbactam at doses of either 50 or 25 mg/kg resulted in bacterial kidney titers that were 2.21 to 2.80 $\log_{10}$ CFU lower than those of the untreated control groups on day 4 ($P < 0.05$). Meropenem efficacy at

TABLE 3 Efficacy of meropenem alone and in combination with vaborbactam against *K. pneumoniae* UNT171-1 in a murine pyelonephritis model[a]

Test	Dose (mg/kg) or day	*n*	Kidney $\log_{10}$ CFU		Change in $\log_{10}$ CFU vs:		*P*
			Mean	SD	Day 4	Meropenem alone	
Meropenem	100	7	3.88	1.55	0.15	NA	0.431 vs untreated day 4; 0.026 vs 300 + 50 mg/kg
	300	6	2.35	0	−1.38	NA	0.021 vs untreated day 4; 0.079 vs 300 + 50 mg/kg
Meropenem + vaborbactam	300 + 50	6	2.48	0.21	−1.25	0.13	0.0317 vs untreated day 4
	100 + 50	7	2.40	0.11	−1.33	−1.48	0.024 vs untreated day 4; 0.218 vs 300 + 50 mg/kg; 0.027 vs 100 mg/kg
	100 + 25	7	2.46	0.2	−1.27	−1.42	0.041 vs untreated day 4; 0.436 vs 300 + 50 mg/kg; 0.017 vs 100 mg/kg
Untreated controls	Day 4	5	3.73	1.44	NA	NA	NA
	Day 5	7	4.05	0.66	0.32	NA	0.280 vs untreated day 4

[a]NA, not applicable.

TABLE 4 Efficacy of meropenem alone and in combination with vaborbactam against *E. coli* UNT167-1 in a murine pyelonephritis model[a]

Test	Dose (mg/kg) or day	*n*	Kidney $\log_{10}$ CFU		Change in $\log_{10}$ CFU vs:		*P*
			Mean	SD	Day 4	Meropenem alone	
Meropenem	100	7	5.78	1.23	−0.84	NA	0.119 vs untreated day 4; 0.003 vs 300 + 50 mg/kg
	300	6	5.8	1.29	−0.82	NA	0.138 vs untreated day 4; 0.004 vs 300 + 50 mg/kg
Meropenem + vaborbactam	300 + 50	6	3.82	0.31	−2.8	−1.98	0.0003 vs untreated day 4
	100 + 50	7	4.11	0.64	−2.51	−1.67	0.0003 vs untreated day 4; 0.197 vs 300 + 50 mg/kg; 0.006 vs 100 mg/kg
	100 + 25	7	4.41	1.38	−2.21	−1.37	0.006 vs untreated day 4; 0.378 vs 300 + 50 mg/kg; 0.07 vs 100 mg/kg
Untreated controls	Day 4	5	6.62	0.98	NA	NA	NA
	Day 5	7	7.09	0.21	0.47	NA	0.162 vs untreated day 4

[a]NA, not applicable.

300 mg/kg was significantly increased with the addition of vaborbactam at 50 mg/kg, resulting in 1.98-$\log_{10}$ CFU lower kidney titers than those with meropenem alone at the same dose ($P = 0.004$).

In this model, meropenem alone was inconsistently effective at reducing the bacterial burden of any of the 3 isolates in the kidneys of infected animals. Vaborbactam alone was not tested in this model, but it has not shown any *in vitro* or *in vivo* activity in previous studies (data not shown).

The benefit of using humanized doses of antibiotics to predict efficacy in complicated urinary infections has been reported (10). Antibiotic plasma levels are more relevant in predicting efficacy than antibiotic kidney levels in complicated urinary tract infections. Therefore, we have used various human exposures of meropenem and vaborbactam to determine the efficacy in a pyelonephritis model. These data indicate that the addition of vaborbactam to the meropenem regimen greatly enhanced the effectiveness of meropenem in reducing the bacterial infection in the kidneys. Overall, the results indicate that the combination of meropenem and vaborbactam may be an effective therapeutic option for the treatment of pyelonephritis due to carbapenem-resistant *Enterobacteriaceae*.

The mouse urinary tract infection model for pyelonephritis was conducted in accordance with the protocols approved by the UNT Health Science Center (UNTHSC)-Institutional Animal Care and Use Committee.

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