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Double-Carbapenem Therapy for Carbapenemase-Producing *Klebsiella pneumoniae*▽

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The limited treatment options available for carbapenemase-producing *Klebsiella pneumoniae* (KPC) have made it a formidable pathogen. Previously we have shown the enhanced activity of pharmacodynamically optimized doripenem against KPC. Capitalizing on KPC's increased affinity for ertapenem, we evaluated the efficacy of a combination of ertapenem and doripenem in both an *in vitro* chemostat and an *in vivo* murine thigh infection model. Overall, the combination of doripenem plus ertapenem demonstrated enhanced efficacy over either agent alone.

The increasing incidence of carbapenemase-producing *Klebsiella pneumoniae* (KPC) is worrisome for a variety of reasons. First, with the addition of this mechanism of resistance these klebsiellae have almost completely exhausted our current arsenal of antimicrobials (9). Moreover, as a result of this multidrug resistance profile, infections related to this pathogen are associated with increased mortality, longer durations of hospitalization, and increased cost of care (8). Previously we have shown that optimized doses of meropenem have the ability to achieve bactericidal activity against KPC with lower MICs, despite the presence of an active carbapenemase, within an *in vitro* chemostat model (3). We have further developed this novel idea of using carbapenems to treat KPC infections by demonstrating that optimized high-dose prolonged infusion of doripenem is able to achieve bacteriostatic activity to modest decreases in bacterial density against KPC with higher MIC values when used in a murine thigh infection model (2).

Ertapenem is considered to be the carbapenem with the least *in vitro* activity against KPC, possibly due to an increased affinity of carbapenemase for this agent (1). We set out to exploit this increased affinity by using this agent in combination with doripenem, which is the most potent carbapenem (i.e., enzyme stability). We tested this combination against KPC in both an *in vitro* chemostat model and in an immunocompetent murine thigh infection model using human-simulated dosing regimens.

Methods. The methodology for the *in vitro* chemostat and the *in vivo* immunocompetent murine thigh infection model has been previously described (2, 3).

Doripenem for injection (Ortho-McNeil-Janssen Pharmaceuticals, Inc., Raritan, NJ) and ertapenem for injection (Merck & Co., Inc., Whitehouse Station, NJ) were used for all studies, with the exception that analytical grade doripenem (Johnson & Johnson Pharmaceutical Research & Development, LLC, Raritan, NJ) was utilized for all *in vivo* studies. All vials were reconstituted with normal saline (NS) according to the manufacturer's instructions immediately before use. Based on potency, analytical grade doripenem was weighed in a quantity sufficient to achieve the required concentration and reconstituted immediately with NS prior to use. All dosing solutions were refrigerated, protected from light, and discarded after 8 h.

Doripenem and ertapenem MIC values for all studies were determined in triplicate by the broth microdilution method as outlined by the Clinical and Laboratory Standards Institute, and the modal MIC value was reported (5). *Klebsiella pneumoniae* isolate KPC 354 (doripenem MIC, 4 μg/ml; ertapenem MIC, 64 μg/ml; modified Hodge test and $bla_{\text{KPC-3}}$ positive) was used for all *in vitro* and *in vivo* studies. Quality control isolate *Pseudomonas aeruginosa* 27853 was used for all MIC testing. KPC 354 was subcultured twice on Trypticase soy agar with 5% sheep blood (BD Biosciences) and grown at 35°C prior to use in all experiments.

In vitro chemostat model. The free doripenem concentrations simulated represented a dose of 2 g every 8 h administered as a 3-h infusion over a 24-h period (i.e., 3 doses) (3). The simulated peak concentration for each dose at 3, 11, and 19 h was 38.4 μg/ml, and the trough at 0, 8, 16, and 24 h was 3.04 μg/ml. The free peak concentration (15 μg/ml) observed after an ertapenem dose of 1 g every 24 h was simulated for the entire 24-h period (7). To simulate a 3-h infusion, doripenem was injected into the model at 30-min intervals from time zero to 3 h while the infusion pump was off. After 3 h, cation-adjusted Mueller-Hinton broth containing ertapenem at 15 μg/ml was continuously pumped into each of the models by a peristaltic pump at a rate that simulated the distribution half-life of doripenem. The doripenem and ertapenem combination models were conducted in duplicate. Samples for bacterial density and doripenem concentration determinations were obtained at multiple time points over the 24-h interval.

In vivo murine thigh infection model. Pathogen-free female ICR mice weighing approximately 25 g were acquired from Harlan Sprague-Dawley, Inc. (Indianapolis, IN), and utilized throughout all experiments. The study was reviewed and ap-

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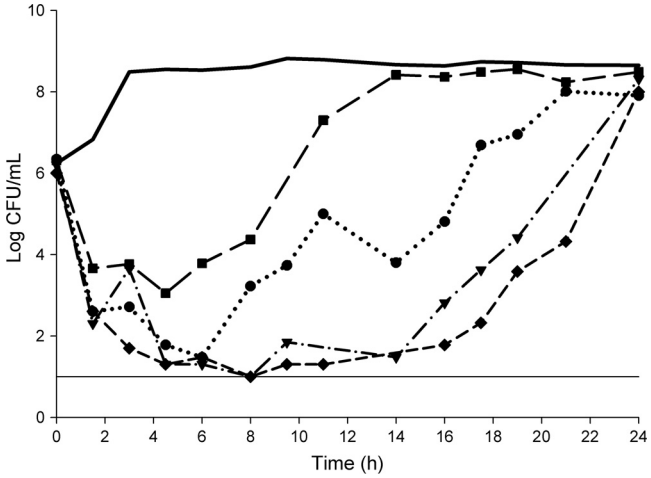

FIG. 1. Bacterial densities of KPC 354 over 24 h in the *in vitro* chemostat model (doripenem MIC, 4 μg/ml). The heavy black solid line represents the control group, the dashed line with squares represents the ertapenem control model (ertapenem alone), the dotted line with circles represents the doripenem control model (doripenem alone), and the dashed lines with either triangles or diamonds represent the doripenem-plus-ertapenem treatment model. The lower limit of detection is set at 10^1 CFU/ml.

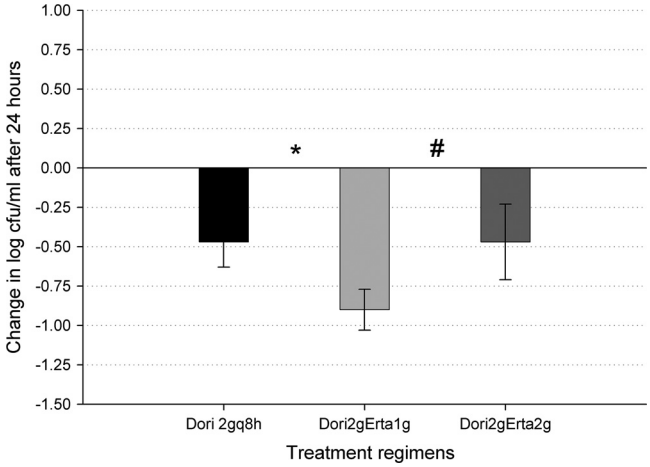

FIG. 2. Comparative efficacies of various dosing regimens of doripenem with or without ertapenem against KPC 354 in the *in vivo* murine thigh infection model (doripenem MIC, 4 μg/ml). The statistical significance of the difference between doripenem alone and doripenem at 2 g plus ertapenem at 1 g is indicated with an asterisk (*), while the statistical significance of the difference between the combination therapies is indicated with a number symbol (#).

proved by the Hartford Hospital Institutional Animal Care and Use Committee (IACUC). Animals were maintained and utilized as approved by the Hartford Hospital IACUC and provided food and water *ad libitum*. The immunocompetent animal infection model was conducted as previously described (2). The free doripenem concentration profile simulated the 24-h cumulative free time above the MIC (*%f*T>MIC) observed in humans given 2 g every 8 h as a 4-h infusion (6), while the free ertapenem concentration profile simulated the *%f*T>MIC observed in humans given 1 g and 2 g every 24 h (7). In order to maximize ertapenem's hypothesized use as a suicide substrate, and in turn maximizing the high affinity of carbapenemase for this agent, ertapenem dosing began 1 h after the initiation of infection while doripenem dosing began 2 h after the initiation of infection. Comparisons of the treatment regimens for efficacy in the *in vivo* model were made using a Student *t* test or a Mann-Whitney U test if the data were not normally distributed. A *P* value of <0.05 was defined *a priori* as statistically significant.

Results. (i) In vitro chemostat model. As assessed by measuring doripenem concentrations, degradation was observed in the infection models (data not shown). Despite this apparent degradation, all doripenem concentration-time profiles were above the MIC for the organism (4 μg/ml) in the majority of the dosing intervals. The average bacterial density of the starting inoculum was $1.73 \times 10^6 \pm 0.47 \times 10^6$ CFU/ml and grew to 4.46×10^8 CFU/ml in the antibiotic free control model after 24 h. Doripenem and ertapenem, alone and in combination, achieved a rapid >3-log reduction in bacterial density within 6 h against KPC 354. Rapid regrowth to control levels was noted in all of the monotherapy regimens, while the combination of doripenem plus ertapenem maintained the >3-log reduction in bacterial density for 16 h before regrowth occurred (Fig. 1).

(ii) In vivo murine thigh infection model. At the start of dosing, 0 h control mice displayed mean bacterial burdens of 6.71 ± 0.23 log CFU/ml. The bacterial load in untreated mice decreased by an average of 0.57 ± 0.33 log after 24 h. All treated and control mice survived to the 24-h sampling point, and the results of the efficacy studies are shown in Fig. 2. The use of doripenem at 2 g every 8 h (q8h) plus ertapenem at 1 g q24h achieved a statistically significant reduction in bacterial density of 0.90 ± 0.13 log CFU/ml versus that of doripenem alone (0.47 ± 0.16 log CFU/ml) ($P < 0.008$). Doubling the ertapenem dose to 2 g did not add any additional decrease in bacterial density to that achieved with doripenem alone, with both the combination and monotherapy regimens producing identical decreases in bacterial density. However, comparison of the different combination regimens showed a statistically significant difference between the combination of doripenem at 2 g q8 h plus ertapenem at 1 g q24 h and doripenem at 2 g q8 h plus ertapenem at 2 g q24 h ($P < 0.006$).

Discussion. An increased incidence of KPC infection in hospitalized patients has been recognized (9). While this pathogen has historically maintained susceptibility to tigecycline and polymyxin B, despite its resistance to all other antimicrobials, resistance to these agents as well has now been reported (4, 10–12). In the face of the ever-more-apparent decreasing armamentarium of commercial compounds with which we can treat KPC infections, attention must be shifted to the use of unlikely combinations of agents that are currently available. In this study, we sought to determine if the use of doripenem, at a level simulating that used for humans, in combination with ertapenem would be efficacious against KPC. To our knowledge, this is the first study in the current literature in which a combination of carbapenems has been tested against KPC in both an *in vitro* and an *in vivo* model.

Within the *in vitro* model data, there is a distinct separation between the results of the monotherapy regimens and those of the combination regimens. By adding ertapenem to dorip-

enem, we were able to maintain a >3-log decrease in bacterial density for 16 h as opposed to 6 h with the monotherapy regimens. All regimens maintained doripenem concentrations above the MIC of the organism (4 μg/ml) for the majority of the dosing interval. This *in vitro* efficacy of the combination therapy translated well into the *in vivo* animal infection model, where once again there was a clear distinction between the monotherapy and combination therapy regimens. *In vivo*, when human-simulated ertapenem at 1 g q24h was added to doripenem at 2 g q8h, statistically significant reductions in bacterial density were seen over that of doripenem alone. The *in vivo* results of this study are in line with previous work by our group which showed that modest decreases in bacterial density can be seen when using doripenem alone against KPC (2).

The enhanced efficacy of the combination regimens over that of the monotherapy regimens in both models may be attributed to the interaction between ertapenem and the carbapenemase enzyme itself. It is well known that ertapenem is the least active carbapenem against KPC, and thus, it is used as an indicator agent to identify the organism (1). We postulate that the enhanced benefit of this combination of carbapenems is related to the enzyme's preferential affinity for ertapenem, due to the ease of hydrolysis versus that of doripenem. Since enzyme is consumed during this interaction with ertapenem, higher concentrations of doripenem are present in the vicinity of the organism than would otherwise be recognized if copious amounts of enzyme were freely available to degrade doripenem. This theory is the rationale for the use of combination carbapenem therapy against these carbapenemase-producing bacteria. The enhanced antibacterial activity seen with the combination regimen in both the *in vitro* and *in viv*o models against this KPC deserves further consideration in the wake of decreasing treatment options against this pathogen. While these early results are encouraging, additional studies are needed to fully evaluate the *in vivo* potential of this combination against additional KPC with a wider range of MIC values.

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