Characterizing In Vivo Pharmacodynamics of Carbapenems against Acinetobacter baumannii in a Murine Thigh Infection Model To Support Breakpoint Determinations

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Pharmacodynamic profiling data of carbapenems for Acinetobacter spp. are sparse. This study aimed to determine the pharmacodynamic targets of carbapenems for Acinetobacter baumannii based on a range of percentages of the dosing interval in which free drug concentrations remained above the MIC (fT>MIC) in the neutropenic murine thigh infection model. fT>MIC values of 23.7%, 32.8%, and 47.5% resulted in stasis, 1-log reductions, and 2-log reductions in bacterial density after 24 h, respectively. The pharmacodynamic targets of carbapenems for A. baumannii demonstrated in vivo are similar to those of other Gram-negative bacteria.

Acinetobacter baumannii, a Gram-negative bacillus with an impressive ability to acquire antimicrobial resistance, has emerged as an important and challenging pathogen in the current health care setting (1, 2). Carbapenems, the most potent of the beta-lactams, possess a broad spectrum of bactericidal activity against Gram-positive and Gram-negative bacteria, a characteristic that is desirable for empirical coverage in health care-associated infections (3). Previous studies have suggested that the pharmacodynamic targets for bacteriostatic and maximal bactericidal activity of carbapenems occur with an fT>MIC of ~20 and ~40%, respectively (4). While extensive work to define these targets has been done in Enterobacteriaceae and Pseudomonas aeruginosa (5–7), no data exist characterizing the pharmacodynamic targets for A. baumannii. Moreover, the breakpoints for carbapenems against Acinetobacter spp. were reassessed at recent Clinical and Laboratory Standards Institute (CLSI) workgroup meetings. However, requests for clinical or animal model data necessary to perform the evaluation rendered no response.

Despite the lack of robust data on their pharmacodynamics, the carbapenems remain an important therapeutic option for serious infections caused by A. baumannii (8). In the current study, we tested the efficacy of three carbapenems (doripenem, meropenem, and imipenem) in a neutropenic murine thigh infection model against A. baumannii isolates to establish a pharmacodynamic target for their antibacterial activity.

Commercially available doripenem (Ortho-McNeil-Janssen Pharmaceuticals Inc., Raritan, NJ), meropenem (Hospira Inc., Lake Forest, IL), and imipenem-cilastatin (Merck & Co., Inc., Whitehouse Station, NJ) were used for all in vivo analyses. Vials were reconstituted and diluted to the appropriate concentrations according to the manufacturer’s instruction. Dosing solutions were stored refrigerated until the time of use and were discarded after 24 h.

Fourteen clinical A. baumannii isolates were used for the in vivo studies. Doripenem, meropenem, and imipenem MICs were determined in triplicate by broth microdilution in accordance with CLSI guidelines (9). Isolates were maintained in double-strength skim milk (BD Biosciences, Sparks, MD) at −80°C. Each isolate was subcultured twice on Trypticase soy agar with 5% sheep blood (BD Biosciences) prior to use.

The protocol was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee. The well-described murine neutropenic thigh infection model was used to determine efficacy (10). Pathogen-free, female ICR mice weighing approximately 20 to 22 g were acquired from Harlan Sprague Dawley, Inc. (Indianapolis, IN), and utilized throughout these experiments. Animals were provided food and water ad libitum. Mice were rendered neutropenic with intraperitoneal injections of 100 and 150 mg cyclophosphamide (Cytoxan; Bristol-Myers Squibb, Princeton, NJ)/kg of body weight, given 1 and 4 days prior to inoculation, respectively. Three days prior to inoculation, mice were given a single 5-mg/kg intraperitoneal injection of uranyl nitrate. This produces a predictable degree of renal impairment to slow drug clearance (11). Two hours prior to the initiation of antimicrobial therapy, each thigh was inoculated intramuscularly with a 0.1-ml solution containing approximately 107 CFU of test isolate.

Beginning 2 h after inoculation, groups of three mice were administered humanized dosing regimens of either 500 mg doripenem intravenously (i.v.) every 8 h as a 1-h or 4-h infusion, 1 g meropenem i.v. every 8 h as a 1-h infusion, or standard imipenem dosing of 55 mg/kg every 8 h using pharmacokinetic data derived from ICR mice in the neutropenic thigh model, as previously developed and validated by our group (6, 7, 12). All therapies were administered over a 24-h period. Doses were administered as 0.2-ml subcutaneous injections. Control animals (three per group) were administered normal saline at the same volume, route, and frequency as the treatment regimen with the most doses per interval. Groups of three untreated control mice were
TABLE 1 Phenotypic susceptibility profiles and corresponding $f/T>MIC$ of Acinetobacter baumannii isolates utilized in the efficacy studies of carbapenems

<table>
<thead>
<tr>
<th>A. baumannii isolate no.</th>
<th>MIC (mg/liter)</th>
<th>% $f/T&gt;MIC$</th>
<th>DOR 1 h</th>
<th>DOR 4 h</th>
<th>MEM</th>
<th>IPM</th>
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</thead>
<tbody>
<tr>
<td>12-13</td>
<td>0.06</td>
<td>0.13</td>
<td>0.13</td>
<td>100</td>
<td>ND</td>
<td>85</td>
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<tr>
<td>1-30</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>75</td>
<td>ND</td>
<td>75</td>
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<td>1-50</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>75</td>
<td>ND</td>
<td>58</td>
</tr>
<tr>
<td>14-12</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>55</td>
<td>82.5</td>
<td>ND</td>
</tr>
<tr>
<td>2-48</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>55</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12-26</td>
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<td>2</td>
<td>42.5</td>
<td>70</td>
<td>58</td>
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<tr>
<td>2-73</td>
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<td>30</td>
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<td>ND</td>
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<td>18</td>
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<tr>
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<td>16</td>
<td>2</td>
<td>10</td>
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<tr>
<td>4-12</td>
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<td>32</td>
<td>32</td>
<td>10</td>
<td>ND</td>
<td>17.5</td>
</tr>
</tbody>
</table>

*ND* represents regimens that were not tested against that particular isolate. **DOR**, doripenem; **IPM**, imipenem-cilastatin; **MEM**, meropenem.

The authors report no financial disclosure relevant to this study.

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We thank Mary Anne Banevicuis, Henry Christensen, Jennifer Hull, Jami Jain, Lucinda Lamb, Debora Santini, Wonhee So, Christina Sutherland, and Pamela Tessier for their assistance with the animal experimentation and research and development (Hartford, CT).

REFERENCES

6. De Ryke CA, Banevicuis MA, Fan HW, Nicolau DP. 2007. Bactericidal activities of meropenem and eteperan against extended-spectrum-beta-lactamases among Gram-negative bacteria. carbapenems are becoming increasingly utilized to treat severely ill patients with nosocomial infections. However, there is a paucity of published data describing the pharmacodynamic parameters required for bacteriostatic and bactericidal effects of carbapenems for Acinetobacter spp. to provide guidance for optimal dosing regimen design. In the current study, carbapenems (doripenem, meropenem, and imipenem) were found to require $f/T>MIC$ values for stasis, 1-log reductions, and 2-log reductions similar to those observed with carbapenems in previous animal infection models against other Gram-negative pathogens (3–7, 13). These data identify $f/T>MIC$ targets of carbapenems for bacteriostatic (24%) and bactericidal (33 to 48%) activity against Acinetobacter spp. and provide guidance for breakpoint setting authorities, such as CLSI and EUCAST.

TABLE 1 Phenotypic susceptibility profiles and corresponding $f/T>MIC$ of Acinetobacter baumannii isolates utilized in the efficacy studies of carbapenems

- **MIC** (mg/liter) for each isolate listed in Table 1.
- **% $f/T>MIC$** for each treatment regimen.
- **DOR 1 h** and **DOR 4 h** represent doripenem concentrations at 1 and 4 hours, respectively.
- **MEM** and **IPM** represent meropenem and imipenem concentrations, respectively.
- **ND** represents regimens that were not tested against that particular isolate.

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REFERENCES


