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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 (*f*T>MIC) in the neutropenic murine thigh infection model. *f*T>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 *f*T>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 10^7 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

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TABLE 1 Phenotypic susceptibility profiles and corresponding *f*T>MIC of *Acinetobacter baumannii* isolates utilized in the efficacy studies of carbapenems[a]

A. baumannii isolate no.	MIC (mg/liter)			% *f*T>MIC			
	DOR	MEM	IPM	DOR 1 h	DOR 4 h	MEM	IPM
12-13	0.06	0.13	0.13	100	ND	85	70
1-30	0.5	0.5	0.5	75	ND	75	ND
1-50	0.5	2	0.5	75	ND	58	55
14-12	1	2	1	55	82.5	ND	50
2-48	1	2	1	55	ND	ND	50
12-26	2	2	2	42.5	70	58	42.5
2-73	2	4	0.5	42.5	70	45	55
12-9	4	8	4	30	52.5	ND	37.5
12-6	8	8	8	20	ND	30	30
12-17	8	8	8	20	ND	ND	30
1-11	8	16	4	20	ND	18	ND
14-6	8	16	16	20	ND	18	25
1-51	16	16	2	10	ND	18	ND
4-12	16	32	32	10	ND	ND	17.5

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

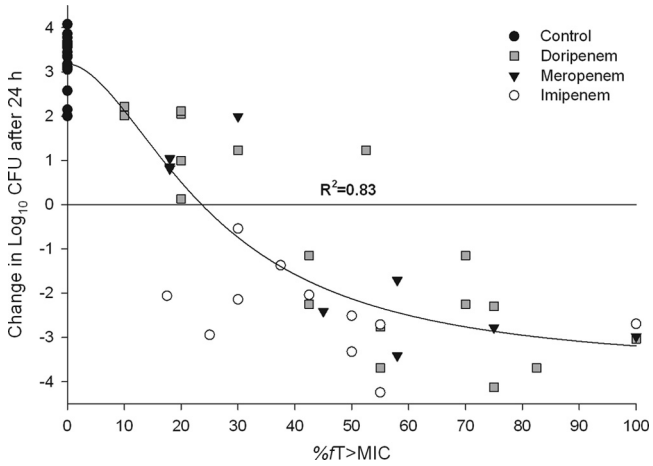

FIG 1 Composite assessment of carbapenem antibacterial effect versus *f*T>MIC for 14 *A. baumannii* isolates in the neutropenic thigh infection model. Symbols represent mean data.

euthanized by CO_2 exposure, followed by cervical dislocation just prior to the initiation of therapy (0 h). All other treatment and control mice were sacrificed 24 h after the initiation of therapy. Mice that did not survive to 24 h were harvested at the time of expiration. Following sacrifice, thighs were removed and homogenized individually in 5 ml of normal saline. Serial dilutions of thigh homogenate were plated onto Trypticase soy agar with 5% sheep blood for determination of bacterial density. Efficacy, defined as the change in bacterial density, was calculated as the change in $\log_{10}$ CFU obtained for carbapenem-treated mice after 24 h. A sigmoid dose-effect model, derived from the Hill equation, was used to calculate *f*T>MIC values of static, 1-log, and 2-log kill for the composite data set.

The phenotypic profiles and respective *f*T>MIC values for the 14 *Acinetobacter baumannii* isolates evaluated are listed in Table 1. The carbapenem MICs for the isolates ranged from 0.06 to 16 μg/ml, 0.13 to 32 μg/ml, and 0.13 to 32 μg/ml against doripenem, meropenem, and imipenem, respectively. A total of 14 control and 38 active treatment groups were evaluated. The mean (±standard deviation) bacterial density for 0-h control mice at the start of dosing was 5.92 ± 0.35 $\log_{10}$ CFU per thigh, which increased to 9.21 ± 0.70 $\log_{10}$ CFU after 24 h. The mice that received treatment all survived to 24 h, while six control mice failed to survive to 24 h. Bacterial densities were similar to those in mice that survived to 24 h and were included in the data analysis. A robust relationship between antibacterial effect and *f*T>MIC (R^2 = 0.83) was observed against *A. baumannii* (Fig. 1). *f*T>MIC values of ≤20% showed minimal activity, with increases in bacterial density for 9 of 10 treatments. An *f*T>MIC of >38% produced reductions in thigh bacterial burden of ≥1 $\log_{10}$ CFU in 22 of 23 treatments. Target *f*T>MIC values needed to achieve a static effect and 1- and 2-$\log_{10}$ CFU reductions for *A. baumannii* are 23.67%, 32.82%, and 47.53%, respectively (R^2 = 0.83). While the current study was designed to be analyzed as a composite data set, when individual agents were evaluated, similar 1-log kill targets (31 to 42% *f*T>MIC) and maximum killing (~4-log reductions) were observed.

With the unceasing progression of drug resistance, particularly 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.

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