Pharmacodynamics of Meropenem against *Acinetobacter baumannii* in a Neutropenic Mouse Thigh Infection Model

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ABSTRACT *Acinetobacter baumannii* infections are difficult to treat and have limited treatment options. Carbapenems, including meropenem, are currently considered the first-line agents for the treatment of infections caused by *Acinetobacter* spp. The percentage of a 24-hour period that the concentration of free drug in plasma is above the MIC (%24-h $fT>$MIC) to achieve stasis, 1 log CFU, or 2 log CFU of bacterial killing against *A. baumannii* has not been studied previously for meropenem. The objective of this study was to determine these parameters for meropenem against *A. baumannii* in a neutropenic mouse thigh infection model. Six *A. baumannii* clinical isolates with MICs ranging from 0.25 to 16 mg/liter were tested. Meropenem produced a bacteriostatic effect with a %24-h $fT>$MIC of 7 to 24% and produced 1 log CFU of bacterial killing with a %24-h $fT>$MIC of 15 to 37%.

KEYWORDS *Acinetobacter baumannii*, meropenem, thigh model

Acinetobacter baumannii colonizes and infects critically ill patients in intensive care units and long-term care units, especially those who are immunocompromised or have chronic and/or underlying disease. *A. baumannii* has as an uncanny ability to survive on environmental surfaces within the hospital setting and can be transmitted via the hands of health care providers and via contaminated tools and equipment (1–4). Currently, there are limited treatment options for *A. baumannii* infection, which has prompted the U.S. Centers for Disease Control (CDC) to consider *A. baumannii* a "serious" antimicrobial resistance threat (5).

Despite the challenges this pathogen poses to the community at large, there are limited scientific data, either *in vivo* or *in vitro*, describing the pharmacodynamics of the limited number of agents that are used to treat *A. baumannii* infections. This absence of data increases the difficulty clinicians face in selecting the optimal dosage regimen for treatment and the prevention of emerging resistance. One such agent, meropenem, is considered one of the first-line agents of choice for the treatment of infections caused by *Acinetobacter* spp.

Despite meropenem being a first-line agent for the treatment of *A. baumannii* infections and in clinical use for over a decade, the exposures required for bacterial killing have only been described in detail for *Enterobacteriaceae* and *Pseudomonas aeruginosa* (6–9). The purpose of these studies was to determine for meropenem the percentage of a 24-hour period that the concentration of free drug in plasma is above the MIC (%24-h $fT>$MIC) required for stasis, 1 log CFU, or 2 log CFU of bacterial killing against *A. baumannii* in a neutropenic mouse thigh infection model.

RESULTS

Susceptibility. The MICs of the *A. baumannii* strains used in these studies are shown in Table 1. Three of the strains were susceptible to meropenem using current breakpoints and dosage regimens, and three were resistant to meropenem.

Pharmacokinetics. The plasma pharmacokinetic parameters for meropenem are shown in Table 2. The total clearances were 1.88, 1.74, and 1.96 liters/h/kg for doses of

TABLE 1 Comparative MICs of *A. baumannii* isolates used in these studies

| Strain | MIC (mg/liter) of[a]: | | | |
	Meropenem	Minocycline	Tigecycline	Levofloxacin
AB1148	0.25	0.03	0.25	0.13
AB1036	0.5	0.06	0.25	4
AB1022	2	1	2	ND
AB1157	4	2	8	16
AB1137	8	8	ND	ND
AB1129	16	4	16	>64

[a]ND, not determined.

30, 100, and 300 mg/kg, respectively. Area under the concentration-time curve (AUC) values increased proportionally with increasing dose and ranged from 15.97 to 153.03 mg · h/liter for 30- and 300-mg/kg single doses, respectively.

Thigh infection model. The bacterial load in the thighs of untreated control mice at the start of treatment for *A. baumannii* strains AB1148, AB1036, AB1022, AB1157, AB1137, and AB1129 was 7.29 ± 0.13, 7.00 ± 0.26, 7.32 ± 0.27, 7.56 ± 0.12, 6.64 ± 0.11, and 7.04 ± 0.12 log CFU/thigh, respectively. All untreated control groups were sacrificed at 12 h postinfection due to physical distress, and bacterial counts were determined. By 12 h, all untreated strains grew by between 1.16 to 2.08 log CFU/thigh.

The individual meropenem %24-h fT>MIC versus change in log CFU/thigh for the six *A. baumannii* strains AB1148, AB1036, AB1022, AB1157, AB1137, and AB1129 are shown in Fig. 1 to 6. Figure 7 shows the pooled data from all six strains.

The % 24-h free meropenem time above MIC for stasis, 1 log CFU, and 2 log CFU of bacterial killing for each strain are shown in Table 3. The meropenem %24-h fT>MIC required to achieve a bacteriostatic effect ranged from 7.3 to 24.2. The meropenem %24-h fT>MIC required to achieve 1 log CFU of bacterial killing ranged from 14.5 to 36.9. The meropenem %24-h fT>MIC to achieve 2 log CFU of bacterial killing ranged from 26.0 to 53.2. The mean meropenem %24-h fT>MIC required to achieve stasis, 1 log CFU, and 2 log CFU of bacterial killing was 17.3, 26.0, and 41.7, respectively.

DISCUSSION

The discovery and development of new treatment options for Gram-negative pathogens has become increasingly difficult and is hampered with a high development cost. Given the limited treatment options available today and the dearth of new treatment options in development, optimizing the dosage regimens of currently available antibacterial agents to maximize the exposure-response relationship is essential.

Recent efforts have been directed toward using pharmacokinetic and pharmacodynamic (PK/PD) parameters to optimize dosage regimens to maximize activity against specific pathogens (10). Meropenem PK/PD have been studied extensively *in vitro*, in animals, and in humans for susceptible and resistant strains of *Enterobacteriaceae* and *Pseudomonas aeruginosa* (6–10). As previously noted by MacVane et al. (11), the PK/PD profile of carbapenems against *A. baumannii* strains is not well defined. In an effort to help define the pharmacodynamics of carbapenems against *A. baumannii*, MacVane et al. simulated 500 mg of doripenem every 8 h by 1- or 4-h infusion, a mouse standardized regimen of imipenem 55 mg/kg every 8 h, and 1 g meropenem every 8 h by 1-h infusion in a neutropenic mouse thigh infection model against 14 *A. baumannii*

TABLE 2 Meropenem pharmacokinetic parameters in Swiss-Webster mice

| Dose (mg/kg) | Pharmacokinetic parameter[a] | | | |
	Total CL (liters/h/kg)	Total AUC (mg · h/liter)	C_{max} (mg/liter)	$t_{1/2}$ (h)
30	1.88	15.97	52.06	0.09
100	1.74	57.57	198.10	0.15
300	1.96	153.03	244.51	0.32

[a]CL, clearance; AUC, area under the concentration-time curve; C_{max}, maximum concentration in plasma; $t_{1/2}$, half-life.
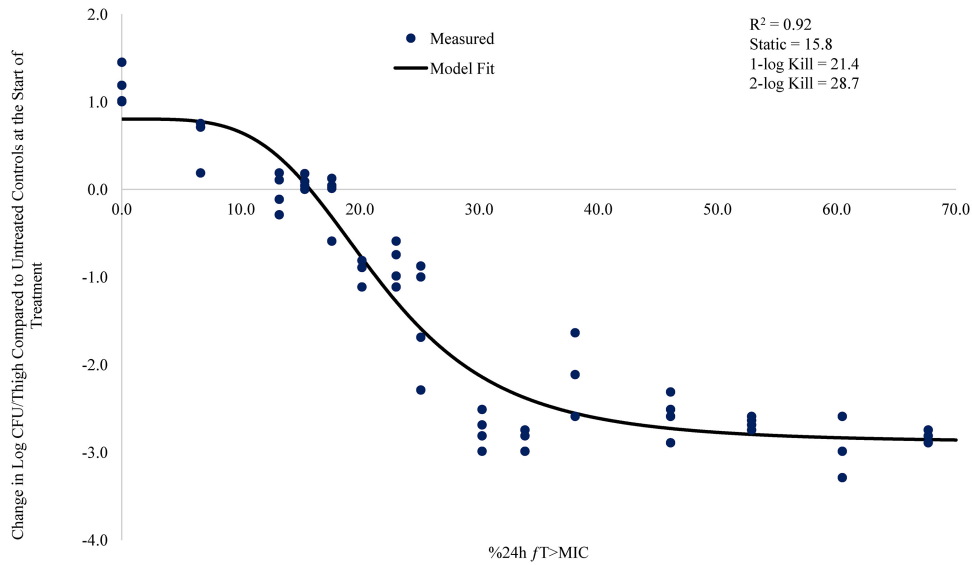
FIG 1 Relationship between meropenem %24-h fT>MIC and change in log CFU/thigh for *A. baumannii* AB1148 (meropenem MIC = 0.25 mg/liter) in a neutropenic mouse thigh infection model.

isolates. They found that the %24-h fT>MIC needed to achieve a static effect and 1-log and 2-log CFU reductions was 23.67, 32.82, and 47.53%, respectively.

While those studies combined the data for three different carbapenems, the current study focused solely on meropenem. In the current study, the meropenem %24-h fT>MIC values needed to achieve a static effect and 1-log and 2-log CFU reductions were 17.3, 26.0, and 41.7, respectively. Overall, the exposure targets and the range of MICs tested are very similar across the two studies. The agreement between the two studies suggests that a PK/PD target for 1 log CFU of bacterial killing can be achieved with free meropenem concentrations above the MIC for 30% of the dosing interval, which is in contrast with the generally accepted PK/PD target for meropenem against other Gram-negative organisms of 40% time above MIC (10). Based on this target, a 0.5-g dose of meropenem administered by a standard 0.5-h infusion every 8 h will
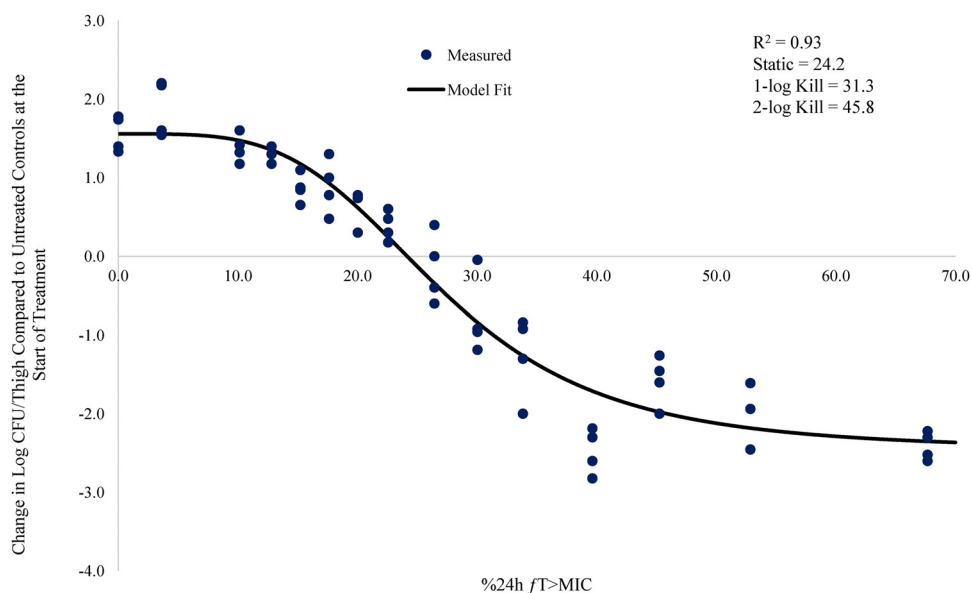

FIG 2 Relationship between meropenem %24-h fT>MIC and change in log CFU/thigh for *A. baumannii* AB1036 (meropenem MIC = 0.5 mg/liter) in a neutropenic mouse thigh infection model.
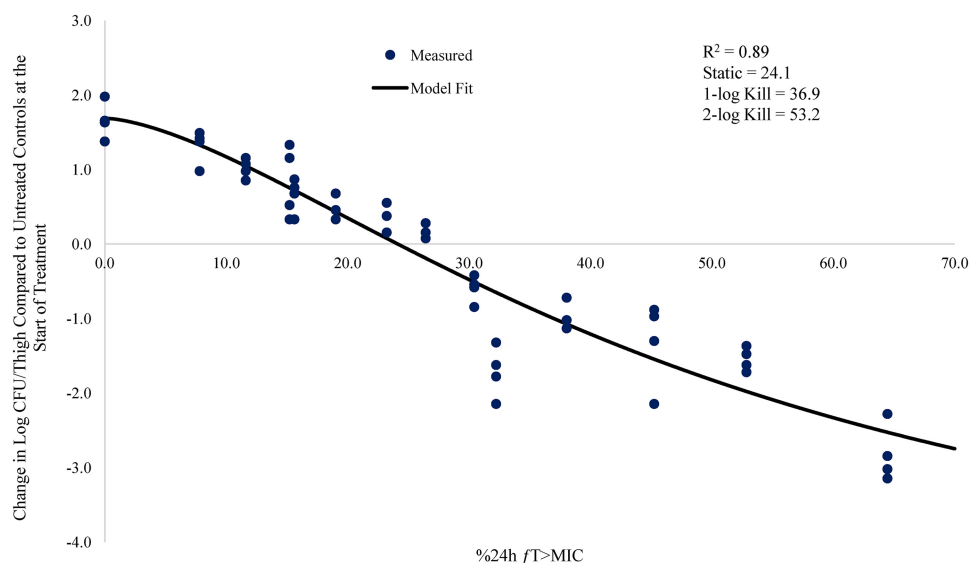
FIG 3 Relationship between meropenem %24-h fT>MIC and change in log CFU/thigh for *A. baumannii* AB1022 (meropenem MIC = 2.0 mg/liter) in a neutropenic mouse thigh infection model.

achieve 30% time above MIC for MICs of up to 4 mg/liter (12) which is 2-fold higher than the current breakpoint (CLSI and EUCAST) for meropenem and *A. baumannii* of ≤2 mg/liter (13, 14). When the dose is increased to 2 g every 8 h by 3-h infusion, free meropenem concentrations were found to be above 16 mg/liter for 30% or more of the dosing interval (12, 15). These data suggest that an optimized meropenem dosage regimen of 2 g every 8 h by 3-h infusion could be effective against *A. baumannii* isolates with MICs of up to 16 mg/liter and that the PK/PD target for *A. baumannii* is lower than that required for other Gram-negative bacteria.

MATERIALS AND METHODS

Animals. All studies using animals were performed under protocols approved by an institutional animal care and use committee (IACUC). Female Swiss Webster mice (5 to 6 weeks of age) were obtained from Envigo (Placentia, CA). Animals were acclimated to laboratory conditions for at least 24 h prior to the initiation of any study.
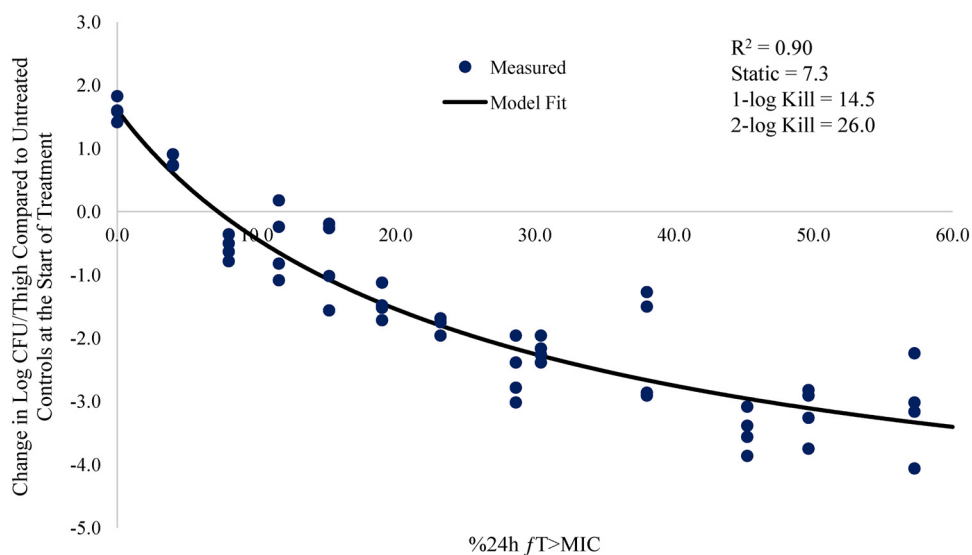

FIG 4 Relationship between meropenem %24-h fT>MIC and change in log CFU/thigh for *A. baumannii* AB1157 (meropenem MIC = 4.0 mg/liter) in a neutropenic mouse thigh infection model.
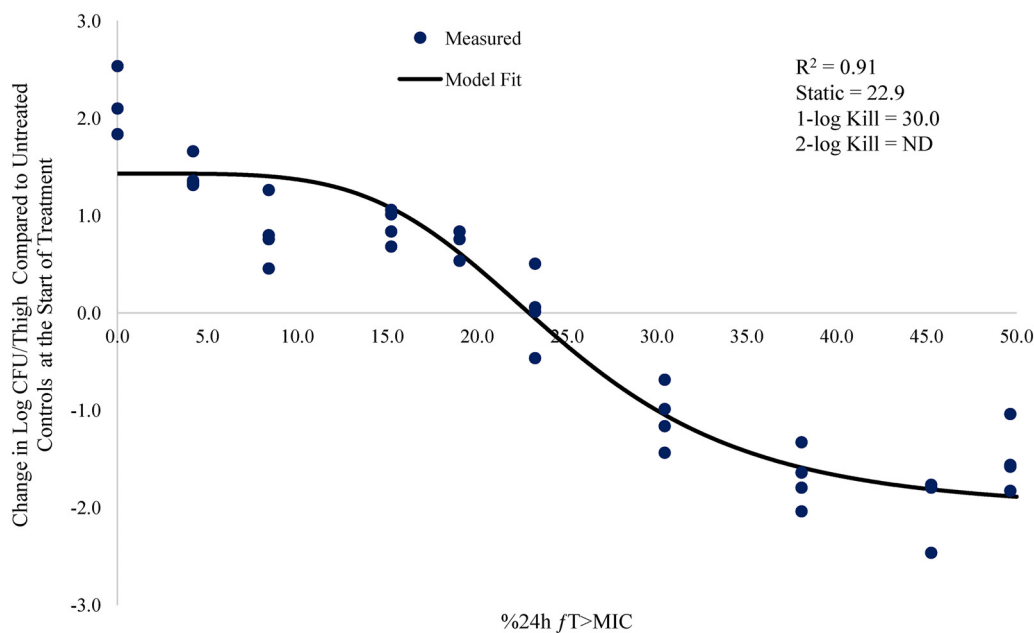
FIG 5 Relationship between meropenem %24-h *f*T>MIC and change in log CFU/thigh for *A. baumannii* AB1137 (meropenem MIC = 8.0 mg/liter) in a neutropenic mouse thigh infection model.

Antimicrobial agents. Meropenem (Sandoz) was reconstituted in 0.9% saline as described in the prescribing information, then further diluted in 0.9% saline to achieve target concentrations.

Strains and susceptibility testing. Six *A. baumannii* clinical isolates were used in these studies. MICs were determined using a broth microdilution assay according to CLSI reference methods (16). Antibiotics were prepared at a concentration equivalent to 2-fold the highest desired final concentration in culture medium and were then diluted directly into 96-well microtiter plates. The microtiter plates were incubated for 17 to 18 h at 37°C and were read by using a microtiter plate reader (Molecular Devices, Sunnyvale, CA) at 600 nm (optical density [OD] value of less than 0.065 = no growth), as well as by visual observation using a reading mirror. The MIC was defined as the lowest concentration of antibiotic at which the visible growth of the organism was completely inhibited.

Pharmacokinetics. The pharmacokinetics of meropenem were determined in uninfected neutropenic mice at doses of 30, 100, and 300 mg/kg. Neutropenia in mice was established by the adminis-
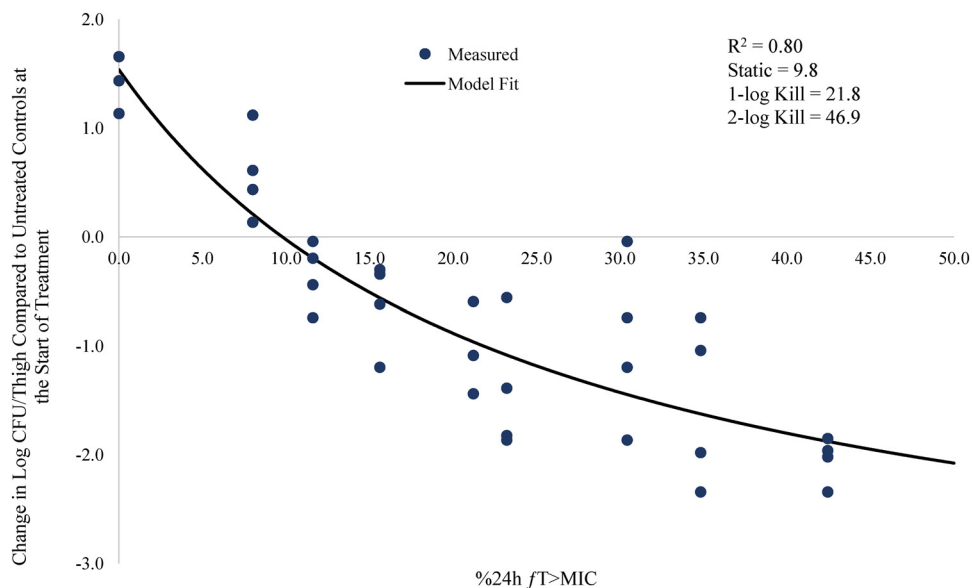

FIG 6 Relationship between meropenem %24-h *f*T>MIC and change in log CFU/thigh for *A. baumannii* AB1129 (meropenem MIC = 16 mg/liter) in a neutropenic mouse thigh infection model.
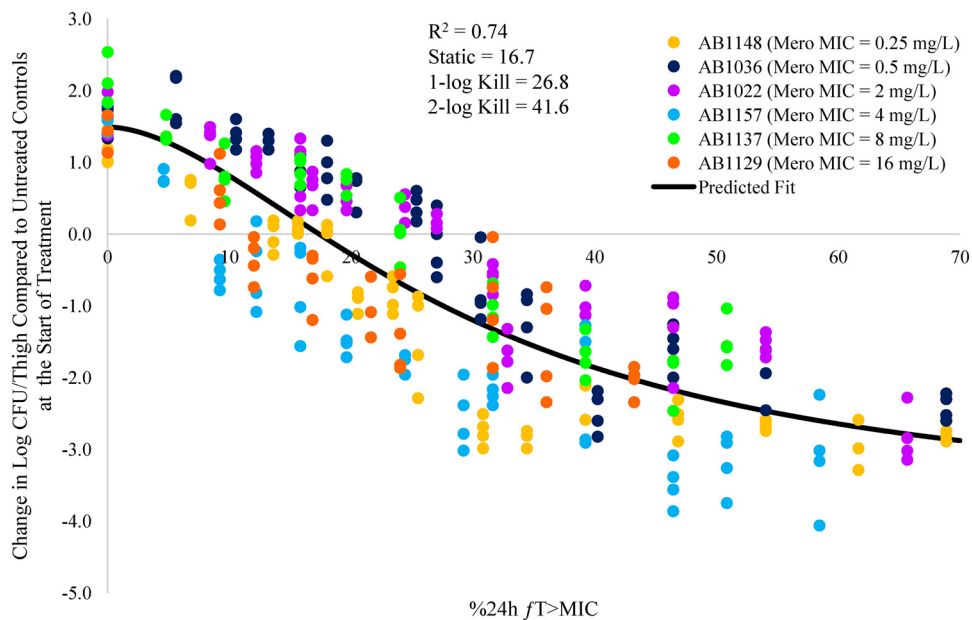
FIG 7 Relationship between meropenem %24-h fT>MIC and change in log CFU/thigh for all six *A. baumannii* strains.

tration of 150 mg/kg cyclophosphamide (Baxter, Deerfield, IL) by the intraperitoneal route 4 days and 1 day prior to the start of the study. At the designated time points, mice were humanely euthanized, and their blood was collected by cardiac puncture and transferred to EDTA-containing tubes. Blood samples were centrifuged within 5 min of collection at 12,000 × g for 5 min to obtain plasma. An equal volume of 1 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 7) was added to plasma samples in order to stabilize meropenem, and samples were stored at −80°C until analyzed.

Bioanalytical assay. Meropenem standard curves were prepared in plasma at concentrations of 0.04 to 50.0 μg/ml. Aliquots (25 μl) of sample were placed in 1.5-ml microcentrifuge tubes containing 200 μl of 4.0 μg/ml doripenem (internal standard) in 10%:45%:45% water-methanol-acetonitrile (vol/vol/vol). The samples were mixed using a vortex mixer, then centrifuged for 10 min at 15,000 × g using a tabletop centrifuge. The supernatant (~150 μl) was removed and added to 400 μl of water in a 96-well plate. The samples were mixed again using a vortex mixer. An aliquot (20 μl) of each sample was injected onto a high-performance liquid chromatography mass spectrometer (HPLC-MS) for quantification. The lower limit of quantitation was 0.04 μg/ml. Plasma concentrations were fitted using a one-compartment, first-order model (Phoenix WinNonlin 8.0; Certara USA, Inc., Princeton, NJ).

Bacterial preparation for thigh model study. Strains were grown in Mueller-Hinton broth (MHB) at 37°C under constant aeration overnight (~20 h). The overnight culture was subcultured into fresh MHB and allowed to regrow for 3 h at 37°C under constant aeration to reach an absorbance of 0.30 to 0.35 at 600 nm (~3 × 10⁸ CFU/ml). The bacterial suspensions were then diluted in fresh MHB to yield ~10⁷ CFU/ml.

Neutropenic thigh infection model. Mice were rendered neutropenic by the administration of 150 mg/kg cyclophosphamide (Sandoz) by the intraperitoneal route 4 days and 1 day prior to the start of the study. Mice were infected by intramuscular injection of 0.1 ml of inoculum (10⁷ CFU/ml) into both thigh muscles while under isoflurane anesthesia (5% isoflurane in oxygen running at 4 liters/min).

Treatment regimens. Starting 2 h postinfection, meropenem was administered every 2, 4, or 6 h over 24 h by the intraperitoneal route. Meropenem was administered at total daily doses ranging from

TABLE 3 Meropenem pharmacodynamic parameters against *A. baumannii* strains[a]

			%24-h fT>MIC to achieve:		
Strain	MIC (mg/liter)	R^2	Stasis	1 log CFU killing	2 log CFU killing
AB1148	0.25	0.92	15.8	21.4	28.7
AB1036	0.5	0.93	24.2	31.3	45.8
AB1022	2	0.89	24.1	36.9	53.2
AB1157	4	0.90	7.3	14.5	26.0
AB1137	8	0.93	22.9	30.1	ND
AB1129	16	0.80	9.8	21.8	46.6
Avg	NA	0.90	17.3	26.0	41.7

[a]NA, not applicable; ND, not determined.

3.75 to 600 mg/kg ($n = 4$ thighs/dose). Meropenem protein binding of 10% was used to calculate free drug (17).

CFU determination. Untreated control animals were euthanized at the start of treatment to establish the baseline bacterial burden. Two hours after the last treatment, treated groups and additional untreated control animals were euthanized via CO_2 asphyxiation. Thighs were aseptically harvested and placed in sterile saline. The tissues were then homogenized using a tissue homogenizer (Pro Scientific, Oxford, CT), serially diluted 1:10 in sterile saline, and plated on Mueller-Hinton agar to determine bacterial counts.

Pharmacodynamic modeling. The relationship between the %24-h $fT>$MIC and the change in log CFU compared to the start of treatment was fitted using the following inhibitory effect (E_{max}) model (Phoenix WinNonlin v 6.3; Certara, Mountain View, CA):

$$E_{max} = E_0 - (I_{max} \times X\gamma)/(X\gamma + IC_{50}\gamma)$$

where E_0 is the effect when X is equal to 0 (i.e., for the untreated control animals), I_{max} is the maximum reduction in the log number of CFU/lung, X is the %24-h $fT>$MIC, IC_{50} is the %24-h $fT>$MIC (X) corresponding to 50% of the maximum bacterial reduction, and γ is the steepness of the curve.

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