Pharmacodynamics of Minocycline against *Acinetobacter baumannii* in a Rat Pneumonia Model

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ABSTRACT Minocycline is currently approved in the United States for the treatment of infections caused by susceptible isolates of *Acinetobacter* spp. The objective of these studies was to determine the minocycline exposures associated with an antibacterial effect against *Acinetobacter baumannii* in a rat pneumonia model. Rats received minocycline doses as 30-min intravenous infusions. In the rat pneumonia model, six clinical isolates of *A. baumannii* with MICs ranging from 0.03 to 4 mg/liter were studied. In this model, minocycline produced a bacteriostatic effect with a free 24-h area under the concentration-time curve (AUC)/MIC ratio of 10 to 16 and produced 1 log of bacterial killing with a free 24-h AUC/MIC of 13 to 24. These exposures can be achieved with the current FDA-approved dosage regimens of intravenous minocycline.

KEYWORDS minocycline, pneumonia, *Acinetobacter baumannii*

Acinetobacter baumannii is the causative agent of many hospital-acquired infections, including pneumonia, ventilator-associated pneumonia, bacteremia, surgical wound site infections, skin and soft tissue infections, meningitis, intra-abdominal abscesses, and urinary tract infections (1–5).

Infections due to *Acinetobacter* have historically been treated with several antibiotic classes, including aminoglycosides, β -lactams, and tetracyclines (1, 4, 6). However, over the last decade, *Acinetobacter* spp. have become increasingly resistant to these drug classes, resulting in these bacteria being among the most threatening organisms in the current antibiotic era (4). Fournier et al. (7) described AbaR1, an 86-kb resistance island containing 45 resistance genes, in a multidrug-resistant (MDR) clinical isolate found in 54 French health care facilities. *A. baumannii* is considered one of the difficult-to-treat ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens, as highlighted by the Infectious Diseases Society of America, and is considered by the U.S. Centers for Disease Control and Prevention to be a serious antimicrobial resistance threat that is likely to worsen without ongoing public health monitoring and prevention initiatives (6, 8).

Currently, carbapenems are regarded as first-line agents for the treatment of infections caused by susceptible *Acinetobacter* spp.; however, increasing carbapenem resistance is reducing the class's clinical utility (4). Minocycline is FDA approved for the treatment of infections caused by susceptible strains of *Acinetobacter* spp. Recent surveillance studies have shown that minocycline is highly active against carbapenem-resistant *A. baumannii* (CRAB) and multidrug-resistant *A. baumannii* (3, 5, 6, 9–11). In two worldwide surveillance studies and samples obtained from patients at Brookes Army Medical Center and Northwestern Medical Center, 90% of *A. baumannii* clinical isolates were susceptible *in vitro* to minocycline, while the rate of carbapenem susceptibility was much lower (11–13). Treatment at Detroit Medical Center found clinical success in 81% of patients infected with CRAB when using intravenous (i.v.) minocycline (11). While these and other studies have demonstrated the utility of minocycline

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TABLE 1 Comparative MICs of *A. baumannii* clinical isolates

Strain	MIC (mg/liter)					
	Minocycline	Tigecycline	Levofloxacin	Amikacin	Gentamicin	Meropenem
AB1148	0.03	0.25	0.13	2	0.5	0.25
AB1036	0.06	0.25	4	8	2	0.5
AB1016	0.25	2	0.5	8	16	1
AB1161	1	0.5	8	4	>64	1
AB1157	2	8	16	8	>64	2
AB1129	4	16	>64	16	>64	16

against MDR *A. baumannii*, the exposures of minocycline required for efficacy against MDR *A. baumannii* have not been well-defined.

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RESULTS

Susceptibility. The MICs of the *A. baumannii* strains used in these studies are shown in Table 1. Three of the strains, AB1161, AB1157, and AB1129, were resistant to at least three antibiotic classes, indicating an MDR phenotype, but all isolates were susceptible to minocycline (MIC range, 0.03 to 4 mg/liter). *A. baumannii* AB1129 was resistant to all antibiotics except minocycline.

Pharmacokinetics. The plasma-versus-time profile of minocycline is shown in Fig. 1. The plasma pharmacokinetic parameters for minocycline are shown in Table 2. The total clearances were 0.29, 0.23, 0.20, and 0.25 liter/h/kg for doses of 10, 30, 60, and 90 mg/kg of body weight, respectively. The areas under the concentration-time curves (AUCs) increased proportionally with increasing dose and ranged from 33.91 to 350.68 mg·h/liter for 10- to 90-mg/kg single doses.

Lung infection model. The bacterial burden in the lungs of untreated control rats at the start of treatment for *A. baumannii* strains AB1148, AB1036, AB1016, AB1161, AB1157, and AB1129 were 8.36, 8.75, 7.71, 7.22, 8.21, and 8.23 log CFU/lung, respectively. Over the 24-h course of the study, these strains grew by 0.84, 1.39, 0.76, 2.50, 1.94, and 1.96 log CFU/lung, respectively. The 24-h AUC/MIC ratios across all strains ranged from 10.61 to 20.12 and 13.10 to 33.93 for static and 1 log of bacterial killing,

FIG 1 Pharmacokinetics of minocycline following a 30-min i.v. infusion in rats.

TABLE 2 Minocycline pharmacokinetic parameters in Sprague-Dawley rats^a

Dose (mg/kg)	Total CL (liters/h/kg)	Total AUC (mg·h/liter)	C _{max} (mg/liter)	t _{1/2} (h)
90	0.25	350.68	113.70	5.11
60	0.20	299.89	90.97	5.11
30	0.23	124.36	44.53	3.04
10	0.29	33.91	10.56	2.05

^aCL, clearance; AUC, area under the concentration-time curve; C_{max}, maximum concentration in plasma; t_{1/2}, half-life.

respectively. The 24-h AUC/MIC ratios for each strain and the pooled data are shown in Table 3. Free minocycline AUC/MIC ratios ranged from 3.5 to 160 for all strains. The free minocycline AUC/MIC ratios for a bacteriostatic effect for AB1148, AB1036, AB1016, AB1161, AB1157, and AB1129 were 10.81, 20.12, 10.61, 16.07, 13.66, and 11.25, respectively. The free minocycline AUC/MIC ratios required to achieve 1 log of bacterial killing for strains AB1148, AB1036, AB1016, AB1161, AB1157, and AB1129 were 19.12, 33.93, 24.22, 18.82, 17.31, and 13.10, respectively (Fig. 2 to Fig. 7). The mean minocycline AUC/MIC ratio required to achieve a bacteriostatic effect was 13.75 ± 3.76 , and the mean minocycline AUC/MIC ratio required to achieve 1 log of bacterial killing was 21.08 ± 7.24 (Fig. 8). Susceptibility studies and visual morphological assessment of the colonies were performed on each strain before and after each efficacy study. No MIC or morphological changes in posttreatment isolates of *A. baumannii* were observed (data not shown).

DISCUSSION

Carbapenems have been considered the treatment of choice for *A. baumannii* infections. Microbiological surveillance trials have reported rates of multidrug resistance, including resistance to carbapenems, of approximately 30% (3). Therefore, as resistance rates continue to rise, antimicrobial stewardship programs must optimize currently available therapeutic options. Minocycline is a proven antibiotic with properties of interest in a time of increasing antibiotic resistance. In 2009, the intravenous formulation of minocycline was reintroduced into the U.S. market to address increasing drug resistance to current first-line agents (3, 6, 9, 10). Minocycline is able to evade most tetracycline efflux pumps, as it is only a substrate of Tet(B) (3, 4, 6, 11, 14–16). Minocycline may also be affected by the cytoplasmic ribosomal protection protein Tet(M) (17). Frequently, suboptimal serum drug concentrations lead to resistance via mutation. Fortunately, intravenous minocycline achieves peak and trough serum concentrations, with standard human doses, that exceed the mutant prevention concentration of 1 mg/liter for *A. baumannii* (1, 4, 6, 11).

Global *in vitro* studies have shown that minocycline is one of the most active antibacterial agents against multidrug- and/or carbapenem-resistant *A. baumannii* isolates (18). The TEST (Tigecycline Evaluation and Surveillance Trial) global surveillance program studied 16,778 *Acinetobacter* clinical isolates, 40% (6,743/16,778) of which were MDR, and minocycline was the most active agent, with susceptibility rates of 84.5% for all strains and 70.3% for MDR strains using the FDA/CLSI breakpoint of 4 mg/liter (18).

We were able to show that minocycline pharmacokinetics are linear and dose proportional in rats. Previous studies of the tetracycline antibiotic class have shown that the free drug AUC/MIC ratio is the pharmacokinetic (PK)-pharmacodynamic (PD) index

TABLE 3 Minocycline pharmacodynamic parameters against *A. baumannii*

Strain	Minocycline MIC (mg/liter)	Free 24-h AUC/MIC for static effect	Free 24-h AUC/MIC for 1-log kill
AB1148	0.03	10.81	19.12
AB1036	0.06	20.12	33.93
AB1016	0.25	10.61	24.22
AB1161	1	16.07	18.82
AB1157	2	13.66	17.31
AB1129	4	11.25	13.10
Mean $\pm$ SD	1.2 ± 1.6	13.75 ± 3.76	21.08 ± 7.24

FIG 2 Relationship between exposure and response for minocycline against *A. baumannii* AB1148.

associated with antibacterial efficacy in experimental models of infection (5, 6). In our rat lung infection model, minocycline produced a bacteriostatic effect against six strains of *A. baumannii* with a 24-h free AUC/MIC ratio of 13.75 ± 3.76 and 1 log of bacterial killing with a 24-h free AUC/MIC ratio of 21.08 ± 7.24 . These data are consistent with previously presented data obtained in an *in vitro* model, where minocycline produced bacterial stasis and a 1-log bactericidal effect against *A. baumannii* with 24-h free AUC/MIC ratios of 16.4 ± 2.6 and 23.3 ± 3.7 , respectively (9).

Based on currently available PK data in healthy human subjects (19, 20) and the

FIG 3 Relationship between exposure and response for minocycline against *A. baumannii* AB1036.

FIG 4 Relationship between exposure and response for minocycline against *A. baumannii* AB1016.

available PK-PD data, minocycline doses of 100 to 200 mg administered twice daily will generate 24-h free minocycline exposures in humans associated with bacterial stasis or killing in susceptible strains (21). A study assessing the safety and pharmacokinetics of high doses of minocycline in healthy subjects is in progress (ClinicalTrials.gov registration number NCT02802631).

FIG 5 Relationship between exposure and response for minocycline against *A. baumannii* AB1161.

FIG 6 Relationship between exposure and response for minocycline against *A. baumannii* AB1157.

MATERIALS AND METHODS

Antimicrobial agents. Minocycline (The Medicines Company) was used for all studies. Minocycline was reconstituted in water and then further diluted in 0.9% saline to achieve the target concentrations. Tigecycline (Pfizer), levofloxacin (LKT Laboratories), amikacin (Fisher), gentamicin (Sigma-Aldrich), and meropenem (Sandoz) were used for susceptibility testing.

Strains and susceptibility testing. Six *A. baumannii* clinical isolates were used in these studies (Table 1). All isolates were confirmed to be *A. baumannii* using an API20NE clinical diagnostic kit (bioMérieux, Marcy-l'Etoile, France).

MICs were determined by a broth microdilution assay according to CLSI reference methods (22) in freshly prepared cation-adjusted Mueller-Hinton broth (23). Briefly, bacterial suspensions were prepared and adjusted to a cell density of 5×10^5 CFU/ml. Antibiotics were prepared at a concentration equivalent

FIG 7 Relationship between exposure and response for minocycline against *A. baumannii* AB1129.

FIG 8 Relationship between exposure and response for minocycline against *A. baumannii*. Solid line, pooled results; orange circles, AB1148; red squares, AB1129; blue circles, AB1016; green triangles, AB1157; teal diamonds, AB1161; purple triangles, AB1036.

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, as well as by visual observation by using a microtiter plate reading mirror. The MIC was defined as the lowest concentration of antibiotic at which the visible growth of the organism was completely inhibited.

Animals. All animal studies were performed under protocols approved by an Institutional Animal Care and Use Committee. Male Sprague-Dawley rats were obtained from Charles River (Hollister, CA). For the pharmacokinetic studies, the rats had surgically implanted femoral and jugular vein cannulas. For the efficacy studies, the rats only had surgically implanted femoral vein cannulas. All surgeries were done by the vendor prior to receipt. Upon receipt, the animals were acclimated to laboratory conditions for at least 24 h prior to the initiation of the study.

Pharmacokinetic studies. Rat pharmacokinetic studies ($n = 3$ rats/dose) were conducted with doses of 10, 30, 60, and 90 mg/kg administered by a 30-min intravenous infusion via the femoral vein cannula. Blood samples were collected from each rat at various time points via a jugular vein cannula. Whole-blood samples were placed in a lithium-heparin microcentrifuge tube and centrifuged for 5 min at 4°C. The supernatants were collected and stored frozen at -20°C for bioanalysis. All data were analyzed using a noncompartmental pharmacokinetic model (Phoenix WinNonlin, v6.3; Certara, Mountain View, CA).

Bioanalytical analysis. Analytical standard curves for minocycline assays were prepared in 10 mM MOPS (morpholinepropanesulfonic acid), pH 7, for concentrations ranging from 0.1 to 100.0 $\mu\text{g/ml}$. Twenty-microliter aliquots of sample were placed in 1.5-ml microcentrifuge tubes containing 20 μl of a 5- $\mu\text{g/ml}$ tetracycline internal standard in 10 mM MOPS, pH 7. Six hundred microliters of precipitant (10% ethanol, 45% acetonitrile, 45% water) was added to each sample and standard. The samples were mixed using a vortex mixer and then centrifuged for 10 min at 4,000 rpm using a tabletop centrifuge. The supernatant was removed and added to 1,000 μl of water in a 96-well plate. The samples were mixed again using a vortex mixer. Twenty microliters of each sample was injected onto a high-performance liquid chromatography-mass spectrometry system for quantification. The coefficient of variation for the quality control was $\sim 2.5\%$.

Bacterial preparation for efficacy study. The bacteria were grown overnight in Mueller-Hinton broth (MHB) at 37°C. On the day of the experiment, the overnight culture was diluted 1:1,000 in fresh MHB and allowed to grow to log phase (~ 3 h). Thirty milliliters of the bacterial suspension was centrifuged at 5,000 rpm for 5 min to obtain a pellet. The supernatant was removed via aspiration, and then the pellet was resuspended with 30 ml of fresh medium. This was centrifuged again at 5,000 rpm for 5 min. The supernatant was aspirated and the pellet was resuspended in 1/10 the volume to obtain a final concentration of 1×10^9 CFU/ml. The bacterial suspension was diluted 10-fold in 20 mg/ml sodium alginate (Sigma-Aldrich, St. Louis, MO) to a final concentration of 1×10^8 CFU/ml.

Lung infection model. Rats were rendered mildly neutropenic using a single dose of cyclophosphamide at 150 mg/kg (Baxter, Deerfield, IL) 2 days prior to the start of infection. On the day of infection, while they were under isoflurane anesthesia, the rats were placed on an angled intubation platform and were infected intratracheally with 0.2 ml of bacterial suspension using a curved oral gavage tip attached to a 1-ml syringe.

Treatment regimens. Minocycline was administered at 2 h postinfection by a 30-min i.v. infusion via the femoral vein cannula. Minocycline was administered every 12 h at total daily doses ranging from 0.46 to 180 mg/kg ($n = 3$ rats/dose). Minocycline protein binding in rat serum of 78% was used to calculate the free drug concentration (24).

CFU determination. Untreated control animals were euthanized at the start of treatment to establish the baseline bacterial burden. At 12 h after the last treatment, the treated groups and additional untreated control animals were euthanized via CO₂ asphyxiation. The lungs were aseptically harvested and placed in 5 ml of 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 the bacterial counts.

Pharmacodynamic modeling. The relationship between the free AUC/MIC and the change in the number of log CFU compared to that at the start of treatment was fit using an inhibitory maximum effect ($E_{\max}$) model (Phoenix WinNonlin, v6.3; Certara, Mountain View, CA) as follows: $E_{\max} = E_0 - (I_{\max} \times X_{\gamma}) / (X_{\gamma} + IC_{50\gamma})$, 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 per lung, X is the free AUC/MIC, IC_{50} is the free AUC/MIC (X) corresponding to 50% of the maximum bacterial reduction, and γ is the steepness of the curve.

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