Pharmacokinetic and Pharmacodynamic Evaluation of the Lantibiotic MU1140

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ABSTRACT: Presented are the pharmacokinetics (PK), exposure–response relationship, and the PK/pharmacodynamic (PD) index predictive of maximum therapeutic efficacy for the lantibiotic MU1140. MU1140, at a dose of 12.5 or 25 mg/kg, was administered intravenously, to characterize its PK parameters in rat. The recently developed *in vitro* PD model of MU1140 activity was enhanced by incorporation of the PK of MU1140 in rat. The linked PK/PD model was used in a simulation study to determine the PK/PD index predictive of *in vivo* efficacy. MU1140 total plasma concentration–time profiles declined biexponentially with elimination terminal half-life of 1.6 ± 0.1 h. Rapid injection of MU1140 was associated with a hypersensitivity reaction that can be blocked by premedication with diphenhydramine. The simulation study revealed that *Staphylococcus aureus* concentrations correlated with $T > \text{MIC}$ making it the PK/PD index best predictive of efficacy. Collectively, these findings suggest that the best route of administration of MU1140 is slow infusion which will increase the time its concentration remains above the MIC, thus maximizing the therapeutic effect and minimizing the observed toxicity. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2521–2528, 2010

Keywords: ADME; antiinfectives; pharmacokinetics; pharmacokinetic/pharmacodynamic models; preclinical pharmacokinetic

INTRODUCTION

Antibiotic resistant bacteria are responsible for a healthcare crisis in the United States and worldwide,[1] where more than 70% of nosocomial infections are caused by these *superbugs*.[2,3] In 2005, infections caused by *Staphylococcus aureus*, which represent 16% of infections nationwide, resulted in 12,000 inpatient deaths, 2.7 million days in excess length of hospital stay, and 9.5 billion dollars in extra hospital charges.[4] Two factors contribute largely to the proliferation of this crisis. First, is the diminishing antibiotics pipeline,[5] especially those with novel mechanisms of action. The second factor is suboptimal dosing, which causes the rapid emergence and spread of new bacterial strains resistant to currently used antibiotics.[6] Only ten antibiotics have been approved by the FDA since 1998,[6] of which only two possess novel mechanisms of action. Thus, new antibiotics and optimal dose and dosage regimen are crucial to contain the crisis at hand.[7]

MU1140 (Fig. 1A) is an antibiotic in preclinical development for the treatment of Gram-positive infections. It is produced by the JH1140 strain of the oral bacterium *Streptococcus mutans*.[8] MU1140 belongs to a group of molecules known as the lantibiotics (lanthionine-containing antibiotics), which are ribosomally synthesized peptides containing various modified amino acids such as lanthionine (Lan; ala-S-ala), methyllanthionine (MeLan; abu-S-ala), didehydroalanine (Dha), and didehydrobuterine (Dhb).[9] These antimicrobial peptides are presumably excreted into the extracellular medium to combat microorganisms competing with the producer bacterium for its habitat. MU1140 has a novel mechanism of action known as *lipid II abduction*, which involves inhibition of peptidoglycan cell wall synthesis by binding to and sequestering the cell wall subunits carrier, lipid II, away from its site of action,[10,11] thus destabilizing the bacterial cell wall and resulting in cell lysis.

In an earlier work,[12] the spectrum of activity of MU1140 was characterized, where it was shown to be a very promising new antibiotic that is active

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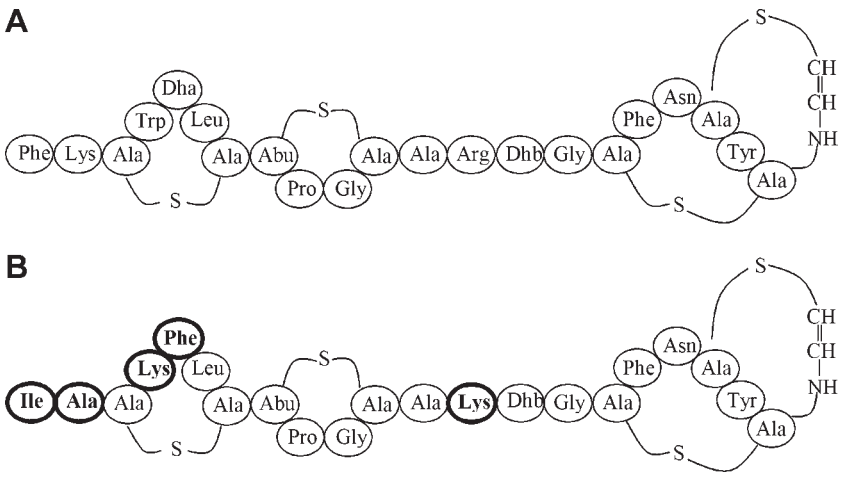

Figure 1. (A) MU1140.[8] (B) Gallidermin.[22] Amino acids different from MU1140 are highlighted.

against all tested multidrug resistant pathogens including methicillin and vancomycin resistant *S. aureus* (MRSA and VRSA, respectively) as well as vancomycin resistant *Enterococcus faecalis* (VRE). MU1140 was shown to be highly bound to serum components (>92%).[13] Further investigation of the effect of serum binding on the dynamics of this lantibiotic revealed that human or rat serum exhibited an inhibitory effect on MU1140 activity against *Streptococcus pneumoniae*. On the other hand, human or rat serum exerted a synergistic effect to MU1140's activity against *S. aureus*. This phenomenon has not been previously reported for any other lantibiotic.[13] The aim of this study was to characterize the pharmacokinetic (PK) of MU1140 in rat. The plasma concentration–time profiles were used to generate a PK model that describes the disposition of MU1140 in the rat. This data are vital for the interpretation of efficacy, toxicity, and safety studies. Time-kill data of MU1140 versus *S. aureus* were used to develop a pharmacodynamic (PD) model of MU1140 activity. The PK and *in vitro* PD models were directly linked to create the PK/PD model predictive of the bacterial load resulting from different MU1140 doses and dosing regimens. Such a model is an initial and essential platform that can be used to rationally design an optimal dose and dosing regimen for future *in vivo* proof of concept studies and clinical trials.

METHODS

Reagents and Antibiotics

Mass spectrometry grade IPA, water, and formic acid were purchased from Sigma (St. Louis, MO). Diphenhydramine for injection was purchased through the University of Florida animal care services from Sigma. Microcon® Centrifugal Devices (10 KDa cut-off) were purchased from Millipore (Bedford, MA). Drug-free, male Sprague–Dawley rat plasma with EDTA was purchased from Rockland Immunochemicals, Inc. (Gilbertsville, PA), aliquoted and stored at −20°C until used. MU1140, produced by fermentation, was manufactured by Oragenics, Inc. (Alachua, FL). Purity (>95%) was confirmed by elemental analysis. The internal standard, Gallidermin was purchased from Alexis Biochemicals (San Diego, CA).

Animals

All animal studies adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985) and were approved by the University of Florida Institutional Animal Care and Use Committee. Jugular vein cannulated male Sprague–Dawley rats (200–220 g, 6–8 weeks old) were purchased from Charles River Laboratories (Raleigh, NC). Animals were housed in temperature and humidity controlled rooms and allowed to acclimatize for 5 days prior to experiment start day. Experiments were conducted at the University of Florida rodent facility (Alachua, FL) where they were given Harlan rat chow (7912) and water *ad libitum*. All rats were weighed immediately before initiation of the study and the weights were recorded and used for dose calculations.

Experimental Design

All animals were subcutaneously injected with diphenhydramine (20 mg/kg) 1 h prior to MU1140 dosing. Each animal was weighed, and the appropriate volume of dose stock solution was brought up to a total volume equivalent to 5 mL/kg rat body weight using saline. The MU1140 dose was administered as a single bolus infusion over a 1-min period. MU1140 PK

parameters were determined in two dose groups, 12.5 and 25 mg/kg rat body weight, with six animals per group. The jugular cannulae were used for dose administration and blood sampling at times −1, 5, 10, 20, 30 min, and 1, 2, 4, and 6 h postdosing. Blood was mixed 10:1 with a 10× anticoagulant stock solution (15 mg/mL sodium EDTA plus 17 mg/mL sodium chloride) and centrifuged at 500*g* for 20 min at room temperature to separate the plasma. Plasma samples were collected immediately and stored at −80°C. Normal saline was used to cleanse the cannulae after MU1140 administration and after blood sampling. Heparinized saline (1000 IU/mL) was used as the cannula lock solution.

Quantification of MU1140 in Plasma

Quantification of MU1140 in rat plasma samples was carried out using the validated LC-MS method developed by Ghobrial et al.[14] Briefly, plasma samples were allowed to thaw at room temperature. Gallidermin was added to a final concentration of 6 μg/mL prior to sample preparation. The sample preparation procedure involved addition of formic acid to a final concentration of 2% (v/v) and vortexing for 30 s. Plasma proteins were precipitated by addition of an equal volume of isopropanol, followed by centrifugation at 16,000*g* for 30 min at room temperature. The supernatant was filtered through a 10 KDa MWCO Microcon filter and the ultrafiltrate was analyzed for its MU1140 content by LC-MS. A Clipeus C-18 analytical column (100 mm × 2.1 mm; 5 μm particle size; Higgins, MA) with a precolumn in-line filter (0.5 μm, MacMod, PA) was used for separation at room temperature. Samples (25 μL) of standards and unknowns were injected onto the column. Proteins were eluted with an acidified (0.3%, v/v formic acid) IPA/water gradient at a flow rate of 250 μL/min. The gradient ranged from 5% to 95% IPA/water (v/v). An API 150 single quadrupole mass spectrometer (Applied Biosystems, Concord, ON, Canada) operated in the positive mode with electrospray ionization was used to detect MU1140 and internal standard (ISTD) using single ion monitoring detection. Quantitation was achieved by monitoring ions at m/z 1133 (MU1140) and m/z 1083 (gallidermin). The standard curve was linear ($r^2 > 0.998$) with 0.039 μg/mL being the lower limit of quantitation and 100 μg/mL was the upper limits of quantitation. The relative standard deviation (RSD) for within-run precision was <15% and bias was <7% of the nominal values.

Noncompartmental PK Data Analysis

WinNonlin version 5.2 (Pharsight Corporation, Mountain View, CA) was used to perform the noncompartmental analysis (NCA) and to calculate the area under the concentration–time curve (AUC), total clearance (CL), half-life ($t_{1/2}$), and volumes of distribution (V_{ss} and V_{area}) of MU1140. $AUC_{0-t_{last}}$ was estimated using the trapezoidal rule. The $AUC_{t_{last}-\infty}$ (the AUC from the last measured time point to infinity) was determined by dividing the last measured concentration by the elimination rate constant of the terminal phase. The total AUC was the sum of $AUC_{0-t_{last}}$ and $AUC_{t_{last}-\infty}$. The clearance was calculated as CL = Dose/AUC. The slope of the terminal phase of the plasma concentration–time profile, λ_z, was estimated from the terminal slope of the log-linear plot of individual plasma concentrations versus time. The terminal half-life ($t_{1/2}$) was calculated using $t_{1/2} = \ln(2)/\lambda_z$. The mean residence time (MRT) was calculated as the ratio of area under the first moment curve (AUMC) divided by AUC, V_{ss} was calculated as CL × MRT and V_{area} was calculated using $V_{area} = CL/\lambda_z$.

Compartmental PK Data Analysis and PK Model

MU1140 plasma concentration–time data for each rat were fitted using a two-compartment open body model described by $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C is the total plasma concentration, t is the time in hours, A is the y-axis intercept for the distribution phase, B is the y-axis intercept for the linear elimination phase. The PK model was selected based on goodness of fit using the Akaike's Information Criterion (AIC),[15] residuals analysis, and by visual inspection of the fitting. The PK model parameters were estimated using WinNonlin's Gauss–Newton algorithm and uniform weighting.

PD Model

The previously described PD model of MU1140 bactericidal activity[13] was fitted to time-kill data of MU1140 versus *S. aureus*. Time-kill studies were performed in triplicates as described earlier.[12] Briefly, bacterial inocula were prepared from *S. aureus* strain ONI33 grown for 4–6 h in Muller Hinton broth (MHB, Becton Dickinson Biosciences, Franklin Lakes, NJ) media and diluted in saline to 0.5 McFarland. Aliquots of the culture were transferred to sterile plastic culture flasks containing MHB. MU1140 was added from a sterile stock solution to final concentrations equal to 0.5, 1, 2, 4, and 8 times MU1140 MIC for *S. aureus*. The assay included a growth control tube with no antibiotic.

The cultures were incubated at 37°C and samples were obtained at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h following addition of MU1140, serially diluted 10-fold in ice cold normal saline and spotted onto duplicate blood agar plates (BAPs). Following incubation at 37°C for 24 h, colonies that arose on plates with 30–300 colonies were counted.

The nonlinear regression software, WinNonlin v. 5.2 (Mountain View, CA), was used to perform the

curve fitting of the PD model to the experimental time-kill data using the Nelder–Meed algorithm. As previously presented,[13] the change in the number of bacteria was calculated using the following differential equation: $\frac{dN}{dt} = N_0[g(N_{max} - N_0/N_{max}) - (k_{max}C/C + EC_{50})]$, where N is the number of bacteria at time t, N_0 is the initial bacterial count, g is the bacterial growth rate constant, N_{max} is the maximum bacterial carrying capacity of the culture, k_{max} (h^{-1}) is the maximum antibiotic kill rate constant, C is the antibiotic concentration at time t, and EC_{50} is the antibiotic concentration that produces half of the maximum effect. N_{max} was obtained directly from the experimental data. The model assume that binding of MU1140 to the bacterial cell wall components is irreversible as suggested by Smith et al.[10] and Smith and Hillman[16] thus causing a decrease in the free and pharmacologically active antibiotic concentration. This was modeled as an elimination process where free MU1140 concentration decreases according to $C = C_0e^{-K_et}$, where C_0 is the initial concentration of MU1140 in the medium and K_e (h^{-1}) is the elimination rate constant of free MU1140 from the culture by irreversible binding to bacterial lipid II.

PK/PD Model, Simulation, and Predictive Index Identification

The PK/PD model (Fig. 4) was assembled by substitution of the concentration term in the PD model with the concentration function from the PK model multiplied by (e^{-K_et}). Mean rat PK parameter were used to generate the predicted *in vivo* MU1140 concentrations used in the PK/PD model. k_{max}, g, and EC_{50} were fixed to the values estimated from the PD model. To identify the PK/PD index ($T > MIC$, C_{max}/MIC, or AUC/MIC) best predictive of therapy's success, the developed PK/PD model was used to simulate the bacterial viable cell counts resulting from different MU1140 doses and dosing regimens. Time-kill data resulting from six different doses (0,12.5, 25, 50, 100, and 150 mg/kg) and eight dosing regimens (1, 2, 3, 4, 6, 8, 12, and 24 doses over a 24 h period) were simulated and bacterial counts at 24 h were used as a marker of the regimen's efficacy. The PK components, C_{max} and AUC, were calculated by simulation of the dosing regimens proposed above using the developed PK model. $T > MIC$ was expressed as the percent of a 24 h period, and for each dose and dosing regimen combination, $T > MIC$ was calculated as the cumulative time the concentration of MU1140 was maintained above the MIC (32 μg/mL).[12]

Statistical Analysis

Statistical analysis of PK parameters was performed using the unpaired Student's t-test where a p-value ≤ 0.05 was considered significant.

RESULTS

Pharmacokinetics of MU1140

The plasma concentration–time profile of MU1140 in Sprague–Dawley rats after a single, 12.5 or 25 mg/kg, IV bolus dose is presented in Figure 2. MU1140 plasma concentration–time profile declined in a

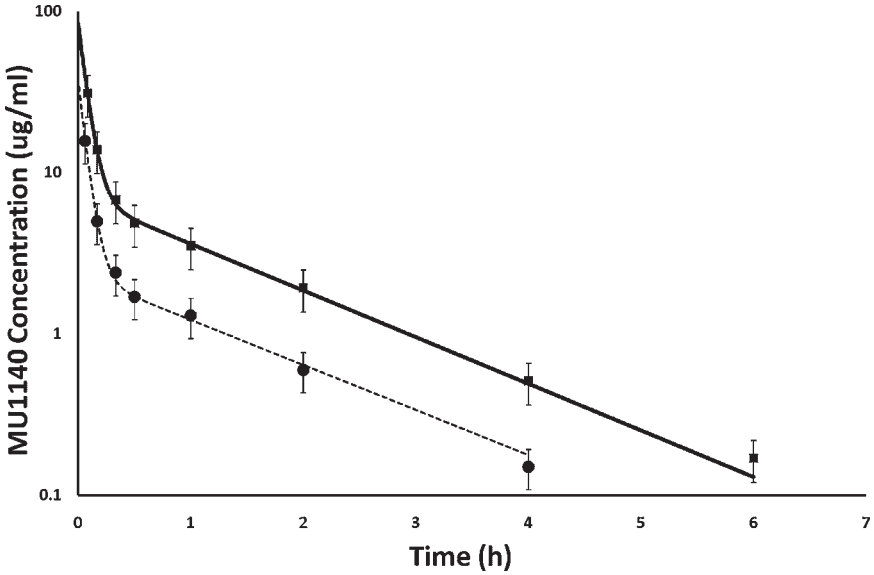

Figure 2. Mean MU1140 PK profile after administration of 12.5 (circles) or 25 (squares) mg of MU1140 per kg rat body weight (symbols). Shown also is the simulated plasma concentration–time profile generated by the PK model (lines). Error bars represent the standard deviation ($n = 6$).

Table 1. Noncompartmental Analysis of MU1140 (12.5 and 25 mg/kg) Concentration–Time Data

	$AUC_{0-\infty}$ (μg h/mL)	CL (L/h/kg)	$t_{1/2}$ (h)	V_{ss}[a] (L/kg)	V_{area}[b] (L/kg)	C_{max} (μg/mL)
12.5 mg/kg	8.7 ± 4.1	1.3 ± 0.21	1.5 ± 0.2	2.2 ± 1.1	2.8 ± 1.03	11.0 ± 4.1
25 mg/kg	19.0 ± 5.5	1.4 ± 0.42	1.7 ± 0.1	2.3 ± 1.0	3.5 ± 1.14	21.0 ± 12.6

PK parameters estimated were AUC, CL, $t_{1/2}$, V_{ss}, V_{area}, and C_{max}. Standard deviation in parenthesis.
[a] V_{ss} is the estimated volume of distribution at steady state calculated as $MRT_{0-\infty} \times CL$, based on the last observed concentration.
[b] V_{area} is the volume of distribution, calculated as CL/λ_z.

biexponential mode and MU1140 was measurable in plasma for up to 6 h postadministration.

Noncompartmental Data Analysis

MU1140 PK parameters estimated by NCA of the plasma-concentration data are summarized in Table 1. The average terminal half-life of MU1140 was 1.6 h. The C_{max} and $AUC_{0-\infty}$ were dose dependent and calculated to be (10.7 ± 4.1, 23.1 ± 12.6 μg/mL), (8.7 ± 4.1, 18.7 ± 5.5 μg h/mL) for the 12.5 and 25 mg/kg dose groups, respectively. Clearance, was dose independent, and was estimated to 1.34 ± 0.21 and 1.44 ± 0.42 L/h/kg for the 12.5 and 25 mg/kg dose groups, respectively. For both dose groups, there was no statistically significant difference between CL, $t_{1/2}$, V_{ss}, or V_{area} (Tab. 2).

Compartmental Data Analysis

MU1140 plasma concentration–time data were fitted using a two-compartment open model equation. The log concentration–time profiles were characterized by a short (<1 h) but clear distribution phase followed by a slower elimination phase, as seen in Figure 2. The mean half-life of the distribution phase ($t_{1/2\alpha}$) was 12 min, while the terminal elimination phase mean half-life ($t_{1/2\beta}$) was 1.3 h. The volume of the central compartment ($V1$) was 0.37 ± 0.23 L/kg and the volume of the peripheral compartments ($V2$) was 1.4 ± 0.56 L/kg. Clearance (CL) and intercompartmental clearance (CLD2) were 1.67 ± 0.56 and 2.55 ± 1.29 L/h/kg, respectively.

Table 2. Compartmental Analysis of MU1140 (12.5 and 25 mg/kg) Concentration–Time Data

V_1[a] (L/kg)	K_{10}[b] (h^{-1})	K_{12}[c] (h^{-1})	K_{21}[d] (h^{-1})
0.37 ± 0.23	9.3 ± 4.9	8.6 ± 3.5	2.4 ± 0.90

PK parameters estimated were V_1, K_{10}, K_{12}, K_{21}. Standard deviation in parenthesis.
[a] V_1 is the volume of the central compartment.
[b] K_{10} rate constant of elimination from the central compartment.
[c] K_{12} rate constant for the transfer from the central compartment to the peripheral compartment.
[d] K_{21} rate constant for MU1140 transfer from the peripheral compartment to the central compartment.

Time-Kill Data and PD Model

The kill profile of MU1140 against *S. aureus* was characterized by a rapid and significant decline (>3 log drop) in bacterial counts within the first 2 h independent of the antibiotic concentration. The experimentally observed and PD model predicted time course of the bacterial concentrations (CFU/mL) are presented in Figure 4. The model accurately predicted the observed bacterial counts for the tested MU1140 concentrations. The EC_{50} of MU1140 for *S. aureus* was 0.01 μg/mL, and the elimination of MU1140 due to binding to bacterial lipid II, K_e, was 2.01 h^{-1}.

PK/PD Model and Simulation

A simulation study was conducted using the developed PK/PD model where the counts of viable *S. aureus* cells were predicted in the presence of MU1140, administered using different doses and dosing regimens. Figure 5 shows the simulated *S. aureus* counts resulting from various MU1140 dosing regimens. A high degree of correlation between $T > MIC$ and the bacterial cell counts at 24 h (Fig. 5A) was observed irrespective of the MU1140 dose or dosing regimen used, suggesting that $T > MIC$ over 24 h is a predictive index for therapy's success.

DISCUSSION

Vancomycin, the first peptide antibiotic to be used in the clinic[17] and the current drug of last resort, is loosing its efficacy against vancomycin resistant *S. aureus*, *E. faecalis*, and other dangerous pathogens. The spread of community and hospital acquired infections due to drug resistant Gram-positive pathogens stresses the need for new antibiotics with novel mechanisms of action. MU1140's unique mechanism of action involves binding and translocation of lipid II away from cell division septa.[10,11] Due to its unique mechanism of action on cell wall biosynthesis, MU1140 is not subject to commonly known mechanisms of antibiotic resistance and thus is active against a wide range of Gram-positive organisms, including MRSA, VRE, and vancomycin intermediate *S. aureus*. In a previous study,[12]

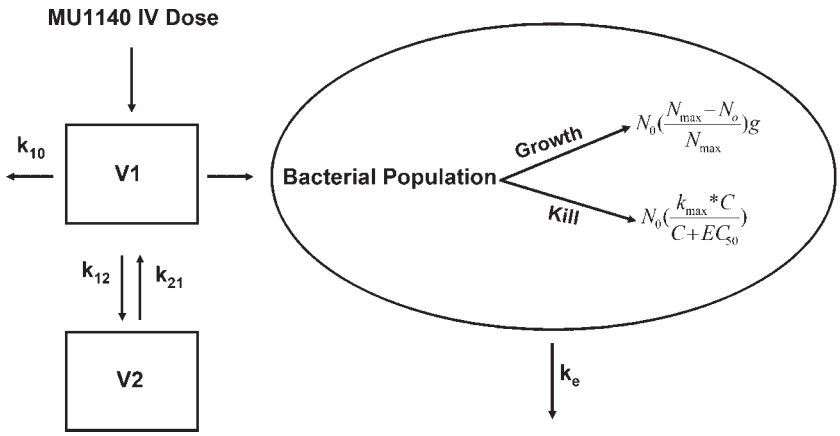

Figure 3. A representation of the exposure–response relationship used to describe the *in vivo* activity of MU1140. *V*1 is the volume of the central compartment, *V*2 is the peripheral compartment. K_{10} is the elimination rate constant from the central compartment; K_{12} and K_{21} are the rate constants for the transfer of MU1140 from the central to the peripheral and from the peripheral to the central compartments, respectively. K_e is the elimination rate constant due to binding to bacterial lipid II.

S. aureus and *S. pneumoniae* were unable to develop significant resistance to MU1140 after continuous subculturing in subinhibitory concentrations of MU1140 for 21 days. If the observed *in vitro* activity is a reflection of its *in vivo* activity, MU1140 would potentially be a very potent antibiotic that could save lives when all other antibiotics fail.

The PK profile of MU1140 in rats was investigated after administration of an intravenous dose equivalent to 12.5 or 25 mg/kg. After intravenous administration of MU1140, its disposition was consistent with a two-compartment open model with a relatively short half-life of 1.6 h. A dose-proportionate increase in the calculated AUC and the observed C_{max} indicates linear PK in the tested dosage range.

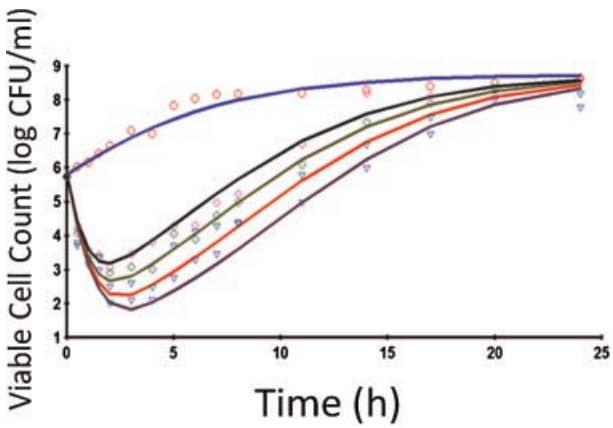

Figure 4. Time-kill studies of MU1140 against *S. aureus*. Observed and predicted *S. aureus* concentrations (CFU/mL). Concentrations used to model the PD activity were 0, 32, 64, 128, and 256 μg/mL.

NCA revealed that the mean volume of distribution of MU1140 in rats was about 3500 mL/kg, which is more than 10 times the rat's extracellular fluid volume.[18,19] This could indicate possible partitioning of MU1140 to blood cells, tissues, and other extravascular sites.

Rapid injection of MU1140 was not well tolerated. A hypersensitivity reaction, similar to a stimulation of histamine release response, is observed within 5 min postadministration of the first dose. This reaction is characterized in rats by redness of the ears and paws, swelling, and lethargy, and usually lasts <20 min, after which the condition subsides. It was also observed that subsequent doses within 24 h do not result in a similar response. Subcutaneous administration of diphenhydramine at a dose equivalent to 20 mg/kg, 1 h before dosing of MU1140, was sufficient to block most of these symptoms. A similar reaction, known as "red man syndrome," was observed when vancomycin was introduced as a rapid infusion.[20] In a clinical setting, premedication with diphenhydramine or administration of MU1140 as a slow infusion might be needed to block or avoid this reaction.

To estimate the activity parameters of MU1140, we conducted time-kill studies of MU1140 against *S. aureus*. The PD model was fitted to the observed bacterial counts and the EC_{50} and K_{max} of MU1140 versus *S. aureus* were estimated. In the model, it was assumed that binding of MU1140 to its target, bacterial lipid II, was irreversible, and therefore the MU1140 concentration in the growth media, was continuously decreasing as the viable bacteria were in mid-log phase and actively multiplying. This phenomenon was modeled as an elimination process of

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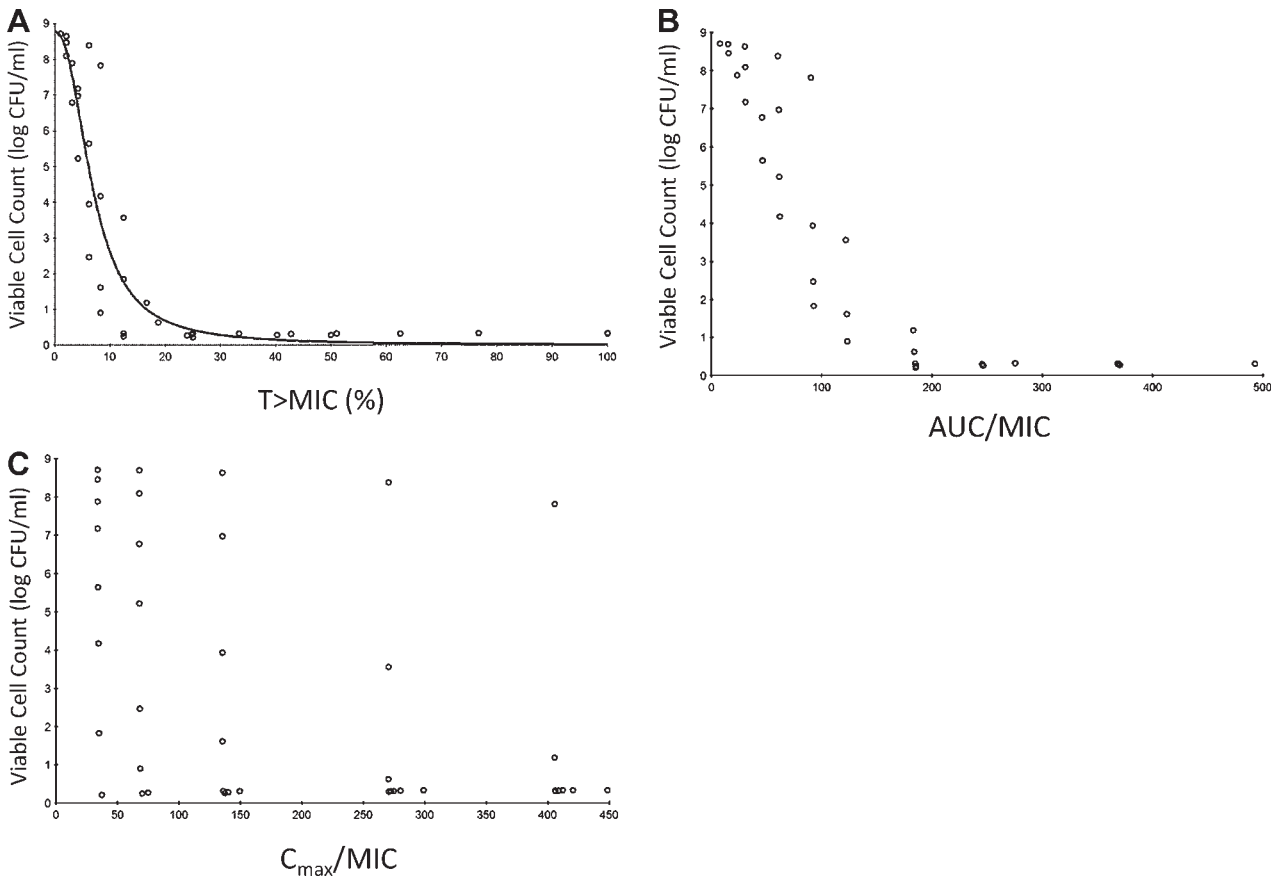

Figure 5. The results of the simulation of *S. aureus* viable cell count resulting from MU1140 doses and dosing regimens. (A) Relationship between MU1140 $T > \text{MIC}$ and predicted viable *S. aureus* counts (round symbols), while the solid line represent the least square regression line. (B) Relationship between MU1140 AUC/MIC and viable *S. aureus* counts. (C) Relationship between MU1140 C_{max}/MIC and viable *S. aureus* counts.

the free and pharmacologically active MU1140 molecules. This hypothesis was based on earlier work[10,16] and is supported by various observations, where it was shown that the concentrations of free MU1140 in the time-kill study growth media has dropped below the limits of detection within few minutes of antibiotic addition (data not shown).

Although the PD model fits the observed data well and can provide very important pathogen and antibiotic specific parameters (e.g., the bacterial growth rate and antibiotic kill rate) but it has limited utility *in vivo* where the antibiotic concentration is changing due to *in vivo* disposition, metabolism, and elimination processes. The PD model was extended by addition of the function that describes the change in MU1140 concentration in a fashion that mirrors the plasma concentration–time profile observed in Sprague–Dawley rats. This hybrid model is capable of predicting the time course and magnitude of the response to MU1140. In the PK/PD model, the simulated bacterial response was driven by the plasma concentrations of MU1140 in the central compartment. This is of physiological relevance since during systemic bacteremia, the pathogen is found in the plasma compartment.[21] Although the model does not take into account the effect of the immune system and its antibacterial activity, it can still be used to correlate different MU1140 doses and dosing regimens with the resulting bacterial load at any time point, and thus a preliminary rational and efficacious dosing regimen could be conceptualized.

In silico, the correlation between $T > \text{MIC}$ and the bacterial cell counts at 24 h was high ($R^2 = 0.91$) and independent of the MU1140 dose or dosing regimen. This is in congruence with the observed minimal concentration-dependent kill behavior of MU1140 against *S. aureus* (Fig. 3). A lower level of correlation ($R^2 = 0.75$) between AUC/MIC and the bacterial counts was observed. This is due to the hybrid nature of the AUC, where a high dose will result in high plasma concentrations and consequently a larger $T > \text{MIC}$. There was no correlation between C_{max}/MIC and the observed bacterial counts. In this simulation study, the linearity of the PK parameters of MU1140 was assumed in the dose range of 12.5–150 mg /kg. This assumption cannot be tested at the current time

due to the unavailability of enough MU1140 to conduct these studies. Due to MU1140's short *in vivo* half-life, constant infusion could be the optimal administration approach, and according to the simulations presented, a $T > \text{MIC}$ of >25% is a starting point during the dose optimization studies since it was sufficient to keep the viable bacterial counts at a minimum.

This study presents the first investigation of the PK of a lantibiotic in a preclinical species. After IV administration of MU1140 in Sprague–Dawley rats, MU1140 showed two-compartment body model plasma kinetics with a $t_{1/2}$ of 1.6 h. Using time-kill data, a PD model was developed to describe the concentration–response relationship of MU1140 against *S. aureus*. The applicability and usefulness of the PD model was enhanced by incorporation of the PK model to create the linked PK/PD model that can be used to simulate theoretical *in vivo* activity time curves under defined assumptions as discussed above. Using this model for simulation purposes, it was shown that $T > \text{MIC}$ correlated well with viable bacterial counts at 24 h, and a $T > \text{MIC}$ of 25% was sufficient to lower the bacterial numbers to its minimum concentration. Further *in vivo* PD studies are required for model optimization.

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