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Human serum binding and its effect on the pharmacodynamics of the lantibiotic MU1140

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ABSTRACT

The degree of MU1140 binding to human serum was measured and the effect of serum on MU1140 pharmacodynamics against *Streptococcus pneumoniae* and *Staphylococcus aureus* was investigated. 92.7% ± 2.0% of total MU1140 was bound to serum components as determined by ultrafiltration when tested in the concentration range 6.25–200 μg/ml.

MIC and time-kill studies were used to study the effect of serum on the dynamics of MU1140. Serum inhibited MU1140 activity against *S. pneumoniae* but was found to enhance its activity against *S. aureus*. This phenomenon has not been reported for any other lantibiotic. Time-kill studies of MU1140 against *S. aureus* in various concentrations of serum revealed that the greatest bactericidal effect was observed at the lowest serum concentration.

Mathematical modeling was used to quantify serum augmentation of MU1140 activity against *S. aureus*. Serum, at the lowest concentration, was shown to decrease MU1140 EC_{50} against *S. aureus* by an order of magnitude.

The data suggests that unbound MU1140 comprise the pharmacologically active fraction. Further, these findings suggest the possible existence of a complex dual inhibition and augmentation effect of serum on MU1140's activity against *S. aureus*. The molecular mechanism responsible for the synergistic action of human serum on MU1140's activity against *S. aureus* remains to be elucidated.

1. Introduction

Lantibiotics are an interesting class of antibiotics defined by the presence of lanthionine (Ala-S-Ala) and other modified and rare amino acids (Chatterjee et al., 2005). Interest in the therapeutic potential of these molecules is increasing as greater understanding about their biology is achieved (Smith and Hillman, 2008). There are approximately 50 known molecules that fall into this class, including MU1140 (Hillman et al., 1998). Like all lantibiotics, MU1140 (Fig. 1A) is ribosomally synthesized and extensively post-translationally modified to its active form (Chatterjee et al., 2005). MU1140 is a 22 amino acid class I type A lantibiotic with a novel mechanism of action known as *lipid II hijacking* (Hasper et al., 2006; Smith et al., 2008). This process involves inhibition of bacterial cell wall synthesis by binding to, abducting, and translocating the peptidoglycan monomers transporter, lipid II, from its normal sites of action in the helical threads along the longitudal axis of the cell (Hasper et al., 2006; Smith et al., 2008).

Knowledge of the interaction of a drug with serum components is essential (Beer et al., 2009) since only the unbound fraction is free to diffuse, reach the biophase, and is pharmacologically active (Tompsett et al., 1947; Craig and Ebert, 1989). If serum binding is not accounted for during dose design, failure of therapy can occur (Kollef et al., 1999) and the potential for emergence of antibiotic resistant bacteria is enhanced (Schmidt et al., 2007). Thus, determining the extent of binding to serum proteins and its effect on the activity of the antibiotic is pivotal for appropriate dose design and successful antibiotic therapy.

The aim of this study was to measure the degree of MU1140's binding to human serum components, and to investigate the effects of serum on MU1140's pharmacodynamic activity against a multidrug resistant *Staphylococcus aureus* strain and a *Streptococcus pneumoniae* strain as measured by MIC determination and time-kill studies. A mathematical modeling approach was used to quantitate the effect of serum on the activity of MU1140 against *S. aureus*. The bactericidal activity of MU1140 against these bacteria has been previously investigated (Ghobrial et al., 2009a,b), but protein binding

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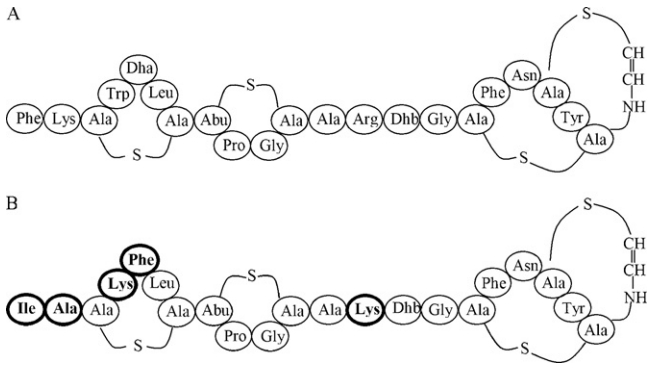

Fig. 1. MU1140 and gallidermin, amino acids specific to gallidermin are highlighted (A) MU1140 (Hillman et al., 1998) and (B) gallidermin.

and bactericidal activity in serum has not been reported for any lantibiotic.

2. Methods

2.1. Antibiotics and reagents

MU1140 was produced by Oragenics Inc. (Alachua, FL) and the bioanalytical internal standard (ISTD), gallidermin (Fig. 1B), was purchased from Alexis Biochemicals (San Diego, CA). MU1140 and gallidermin stock solutions were prepared in 1:1 (v/v) mixture of isopropyl alcohol (IPA):water at a concentration of 25 μg/ml and stored at −80 °C until used. Mass spectrometry grade IPA, water, and formic acid were purchased from Sigma (St. Louis, Mo). Nunc™ 50 ml vented cap tissue culture flasks with canted necks used in the time-kill studies were purchased from Nunc A/S (Roskilde, Denmark) and Microcon® Centrifugal Devices (10 kDa cutoff) were purchased from Millipore (Bedford, MA). Drug-free human serum (serum) was purchased from Rockland Immunochemicals (Gilbertsville, PA). Serum was used either untreated or following heat inactivation by incubation in a 55 °C water bath for 30 min. Aliquots were stored at −20 °C until used. Cation adjusted Mueller–Hinton Broth (MHB) and Todd Hewitt Broth (THB) were purchased from Difco (Detroit, USA). Blood Agar Plates (BAPs) were purchased from Remel Microbiology Products (Lenexa, KS, USA).

2.2. Bioanalytical standards and quality control samples

Working solutions of MU1140 (1 μg/μl) and gallidermin (0.1 μg/μl) were prepared in 10% IPA. Calibration standards of MU1140 were prepared in serum filtrate by dilution of MU1140 working solution to an initial concentration of 100 μg/ml followed by nine serial 2-fold dilutions, to yield standards with the following concentrations; 100, 50, 25, 12.5, 6.25, 3.25, 1.13, 0.63, and 0.33 μg/ml. The serum filtrates used in this step were prepared by centrifugation of serum in Microcon Centrifugal Devices at room temperature for 1 h at 1000 × *g*. The quality control (QC) samples were also prepared using serum filtrate at 3 concentrations, low (1 μg/ml, LQC) medium (10 μg/ml, MQC), and high (50 μg/ml, HQC).

2.3. Determination of protein binding

The degree of binding of MU1140 to serum components was measured in the range of 6.25–200 μg/ml. Heat inactivated and untreated pooled human serum samples were spiked with MU1140 working solution to yield a final concentration of 200 μg/ml, which was serially 2-fold diluted to 6.25 μg/ml. The samples were incubated at 37 °C for 1 h, after which an aliquot was transferred to the Microcon Centrifugal Devices and centrifuged at 1000 × *g* for 1 h at 37 °C. Standards and QC samples were treated in the same fashion. ISTD working solution was added to the filtrates for a 6 μg/ml final concentration. To determine the degree of MU1140 binding to the Microcon Centrifugal Devices, samples of serum filtrates were spiked with MU1140 and filtered. All samples were analyzed by LC-MS as described below. The unbound fraction was estimated from the ratio of drug concentration in the filtrate to that in the original serum samples.

2.4. Equipment and analysis conditions

To quantitate the MU1140 content in the samples, the previously published LC-MS method (Ghobrial et al., 2009a,b) was used. Briefly, the LC-MS analysis system used consisted of a Surveyor plus autosampler and pump (ThermoFisher Scientific, San Jose, CA) coupled to an API SCIEX 150EX single quadrupole mass spectrometer (Concord, ON, Canada). Separation of sample components was achieved by using a Clipeus C-18 analytical column (100 mm × 2.1 mm, 5 μm particle size; Higgins, MA, USA) with a pre-column in-line filter (0.5 μm, MacMod, PA) at room temperature. 25 μl samples of standards and unknowns were injected onto the column. Column contents were eluted using an acidified (0.3% (v/v) formic acid) IPA:water gradient flowing at a rate of 250 μl/min and the gradient ranged from 5% to 95% IPA:water (v/v). Ions were generated by electrospray ionization and detected in the positive mode. The ion source temperature was maintained at 475 °C and a voltage of 5.5 kV was applied to the sprayer needle. Nitrogen was used as the nebulizer and curtain gas. MU1140 and gallidermin were detected using single ion monitoring (SIM) and the Analyst software 1.4 (Concord, ON, Canada) was used for data collection and integration of the chromatographic peaks. The peak area ratios of analyte to ISTD were plotted as a function of MU1140 concentration in standard solutions. A linear curve fit without weighting was used to generate the regression line. The concentrations of MU1140 and gallidermin in the QC samples and unknowns were computed using the regression equation of the calibration curve.

2.5. Broth preparation

Heat inactivated as well as untreated human serum was used in these and subsequent studies. MHB was used to grow *S. aureus* strain ONI133 and THB was used to grow *S. pneumonia* strain ATCC 49619. Both media were prepared at four times (4×) the manufacturer's suggested concentration and autoclaved prior to use at 121 °C (15 min per 1 l). Serum-containing media were prepared as follows: 0% serum medium contain one part 4× broth and three parts sterile 0.9% sodium chloride; 25% serum medium contain one part 4× broth, one part serum, and two parts 0.9% sodium chloride; 50% serum medium contain one part 4× broth, two parts serum, and one part 0.9% sodium chloride; and the 75% serum medium contain one part 4× broth and three parts serum.

2.6. Bacterial cultivation

The bacterial inocula were prepared from colonies grown overnight on BAP at 37 °C in ambient atmosphere supplemented with 5% carbon dioxide. Cells were scraped from the plate using an inoculation loop and suspended in sterile normal saline solution. Turbidity was adjusted to 0.5 McFarland units using a nephalometer (BD Biosciences, Franklin Lakes, NJ), which is equivalent to a concentration of 1×10^8 colony forming units per milliliter (cfu/ml).

2.7. MIC studies

A microdilution method in 96-well microtiter plates was employed using either neat broth or broth supplemented with 25% or 50% (v/v) serum to determine MU1140's MIC for *S. aureus* and *S. pneumonia*. Each well was inoculated with 5×10^5 cfu/ml of bacteria into broth (with or without serum) containing serial 2-fold dilutions of MU1140 ranging from 0.025 to 12.8 μg/ml, namely 0.025, 0.05, 0.10, 0.20, 0.40, 0.8, 1.6, 3.2, 6.4, 12.8 μg/ml. Following incubation for 18–24 h at 37 °C in an atmosphere enriched with 4–6% carbon dioxide, the MIC was determined as the lowest concentration at which there was no visible turbidity. The experiment was conducted in triplicates.

2.8. Time-kill studies

Two types of time-kill studies were conducted: studies in which the amount of serum in the growth medium was fixed at 50% and the concentration of MU1140 varied (0.1–16 × MIC); and studies in which the initial concentration of MU1140 (0.5 × MIC) was the same for all groups and the amount of serum in the growth media was varied (0%, 25%, 50%, and 75%). For all time-kill studies, an *in vitro* model consisting of 8 Nunc™ 50 ml vented cap tissue culture flasks, each containing 20 ml of the appropriate broth media, were used. A 100 μl aliquot of the bacterial inoculum was added to each flask containing 20 ml of the appropriate medium to produce a final inoculum of approximately 5×10^5 cfu/ml. The bacteria containing flasks were incubated standing for 2 h at 37 °C in an atmosphere of 5% CO_2 to allow them to achieve exponential growth before adding the appropriate amount of MU1140. The MU1140 concentrations tested for each bacterial strain was based on previously determined MIC values (Ghobrial et al., 2009a,b). At least six different concentrations were investigated besides the MIC, which included subinhibitory (0.06, 0.12, 0.25 and 0.5 × MIC) and suprainhibitory (2, 4, 8 and 16 × MIC) concentrations. A control experiment with bacteria and no drug was performed in parallel. Fifty microliter samples were removed at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, and 24 h post-antibiotic addition, diluted in saline, and spotted on BAPs for quantification as described below. All time-kill studies were conducted in triplicates.

2.9. Bacterial quantification

Plates were divided into four quadrants. Five 10 μl droplets of each dilution were spotted equidistantly onto one of the quadrants. Duplicate spots were plated onto an adjacent quadrant and, after air drying, the plates were incubated at 37 °C in 5% CO_2 for 18–24 h. Following incubation, colonies that arose were counted. Data were used from quadrants that contained 30–300 colonies, and was plotted using Microsoft Excel®. Positive controls with bacteria but no drug were run simultaneously. The experiments were performed independently in triplicate. The means and standard deviations were calculated.

2.10. PK/PD model

The nonlinear regression software, WinNonlin v. 5.2 (Mountain View, CA), was used to perform curve fitting of the PK/PD model to the experimental time-kill data using the Nelder–Meed algorithm. The change in the number of bacteria was calculated using the following differential equation:

$$\frac{dN}{dt} = N_0 \left[\left(g \frac{N_{max} - N_0}{N_{max}} \right) - \left(\frac{k_{max} \times C}{C + EC_{50}} \right) \right]$$

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 assumption of the model was that binding of MU1140 to the bacterial cell wall components is irreversible as suggested by Smith and Hillman (2008) and Smith et al. (2008), thus causing a decrease in the free and pharmacologically active MU1140 concentrations. Free MU1140 concentrations were calculated using $C = C_0 e^{-ket}$, 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 due to the irreversible binding to bacterial lipid II. In order to compare the k_e and EC_{50} in the presence or absence of serum, g and k_{max} were estimated in the absence of serum and fixed during the fitting of bacterial counts in the presence of the various serum concentrations. The goodness-of-fit was assessed by visual inspection and residuals analysis.

3. Results

3.1. Determination of the degree of binding of MU1140 to human serum

The average MU1140 percentage binding to serum was concentration independent when tested in the range of 6.25–200 μg/ml and was determined to be 92.7% ± 2.0%. MU1140 content of unfiltered serum filtrate samples indicated that the antibiotic's binding to the ultrafiltration device was below the limit of detection (data not shown). In addition, there was no difference in the degree of binding to heat inactivated versus untreated serum (data not shown).

3.2. Effect of protein binding on the in vitro activity of MU1140: MIC studies

The effect of heat inactivated human serum on the *in vitro* activity of MU1140 against *S. pneumoniae* strain ATCC 49619 and *S. aureus* strain ONI 133 was studied by measuring the MICs of MU1140 for these microorganisms in the presence and absence of various serum concentration. The presence of 25% and 50% (v/v) serum caused a 2-fold and 4-fold increase in MU1140 MIC, respectively, against *S. pneumoniae*. Conversely, the presence of 25% and 50% serum increased MU1140 activity against *S. aureus* as indicated by a serum concentration-independent 4-fold reduction in the MIC (Table 1). Identical results were obtained when this experiment was repeated using untreated serum (was not heat inactivated).

3.3. Effect of protein binding on the in vitro activity of MU1140: time-kill studies

To investigate MU1140's kill profile in the presence of heat inactivated serum, time-kill studies of MU1140 against *S. pneumoniae* and *S. aureus* were conducted in serum containing broth (50%).

Table 1
MU 1140 MICs for *Streptococcus pneumoniae* (ATCC 49619) and *S. aureus* in 0%, 25%, and 50% human serum. Standard deviations presented in parenthesis.

Organism	MIC (μg/ml) 0% serum	MIC (μg/ml) 25% serum	MIC (μg/ml) 50% serum
S. pneumoniae[a]	0.8	1.6	3.2
S. aureus[a]	6.4	1.6	1.6

[a] $n = 6$.

In the presence of 50% serum, MU1140 antibacterial activity was decreased against *S. pneumonia*. When compared to cell counts in the absence of serum (Fig. 2A), a substantial increase in viable bacterial counts was observed at MU1140 concentrations below 4 × MIC in the presence of serum (Fig. 2B).

The viable cell counts in the presence versus absence of serum as a function of each different MU1140 concentration is presented to more clearly demonstrate the effect of serum on the interaction of the antibiotic with *S. pneumoniae* (Fig. 3, panels A–H). The figure clearly demonstrates the inhibitory effect of the presence of serum on the activity of MU1140 at concentrations below 4 × MIC. MU1140 activity seemed comparable in the presence and absence of serum at concentrations equivalent to and above 4 × MIC for *S. pneumoniae*.

Serum exerted the opposite effect on the MU1140 activity against *S. aureus* strain ONI133. The *S. aureus* viable cell count decreased substantially in the presence of 50% serum when compared to viable cell counts in the absence of serum. Fig. 4A and B illustrate the differences in bacterial cell counts in the presence and absence of serum, respectively.

Again, to more clearly demonstrate the effect of serum on MU1140's activity against *S. aureus*, the viable cell counts in the presence versus absence of serum as a function of each different MU1140 concentration was co-plotted in separate panels (Fig. 5, panels A–H). An obvious augmentation effect due to serum was observed at MU1140 concentrations below 4 × MIC.

To further understand the correlation between the effect of serum on the activity of MU1140 against *S. aureus*, a second set of time-kill studies were performed in which the bacterial inoculum was fixed at 5×10^5 cfu/ml, MU1140 concentration applied was 0.5 × MIC, but the serum concentration was varied (0%, 25%, 50%, and 75%). As shown in Fig. 6, the presence of serum at low levels had the most drastic effect on the bacterial counts, followed by 50% and 75% serum, which were comparable. The highest numbers of *S. aureus* counts were observed in the 0% serum flask. Addition-

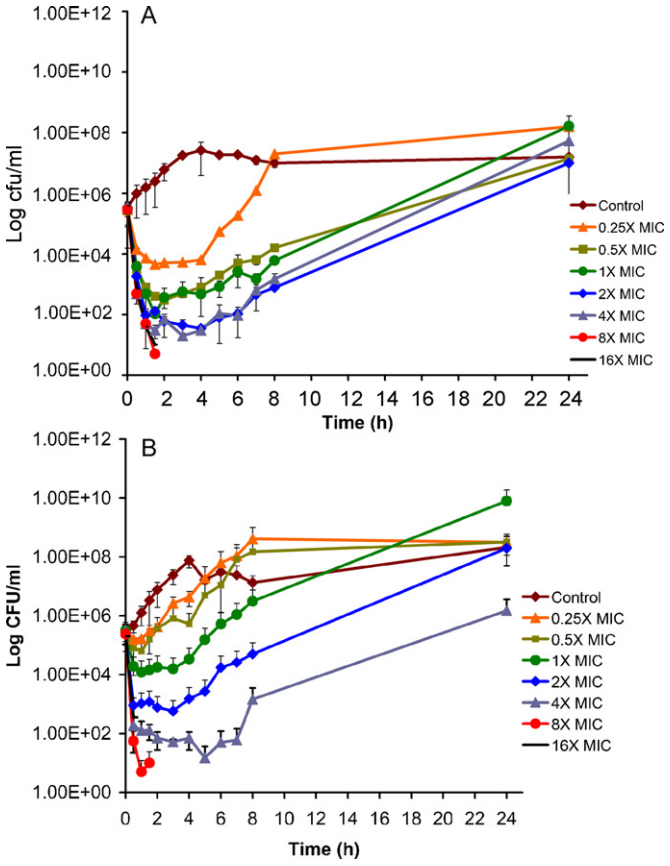

Fig. 2. Time-kill studies of MU1140 against *S. pneumoniae*. (A) Time-kill studies in broth and (B) time-kill studies in 50% serum.

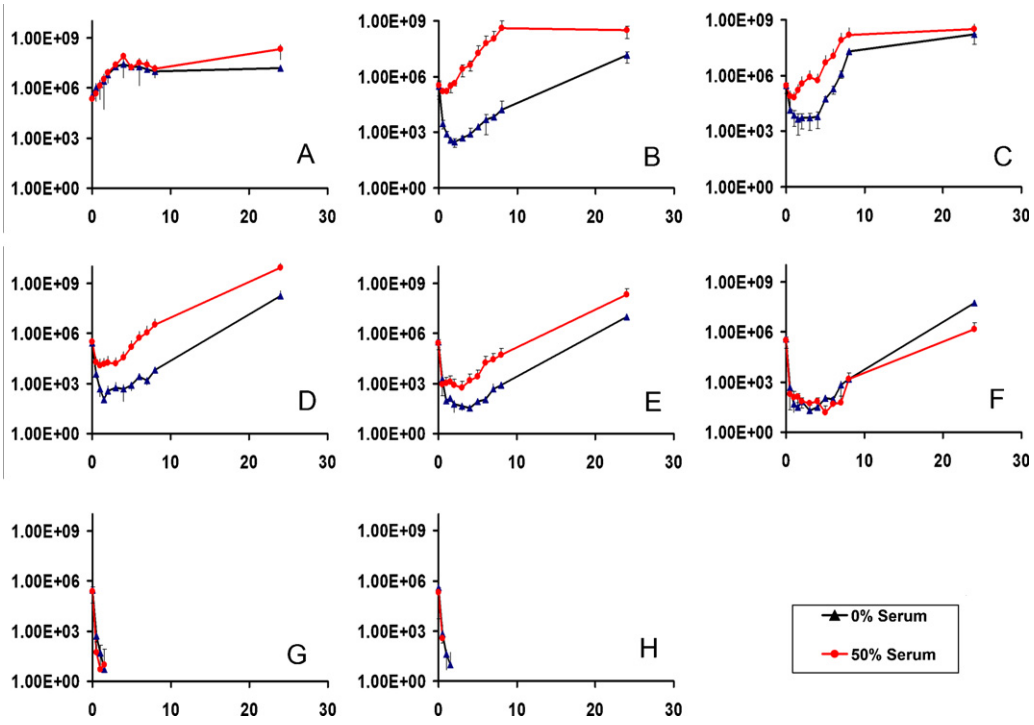

Fig. 3. Side-by-side *S. pneumoniae* counts in flasks containing broth versus 50% serum and equivalent MU1140 concentrations. Log cfu/ml was plotted on the *y*-axis and time in hours was plotted on the *x*-axis. (A) Growth control, no MU1140. (B) 0.25 × MIC, (C) 0.5 × MIC, (D) 1 × MIC, (E) 2 × MIC, (F) 4 × MIC, (G) 8 × MIC, and (H) 16 × MIC.

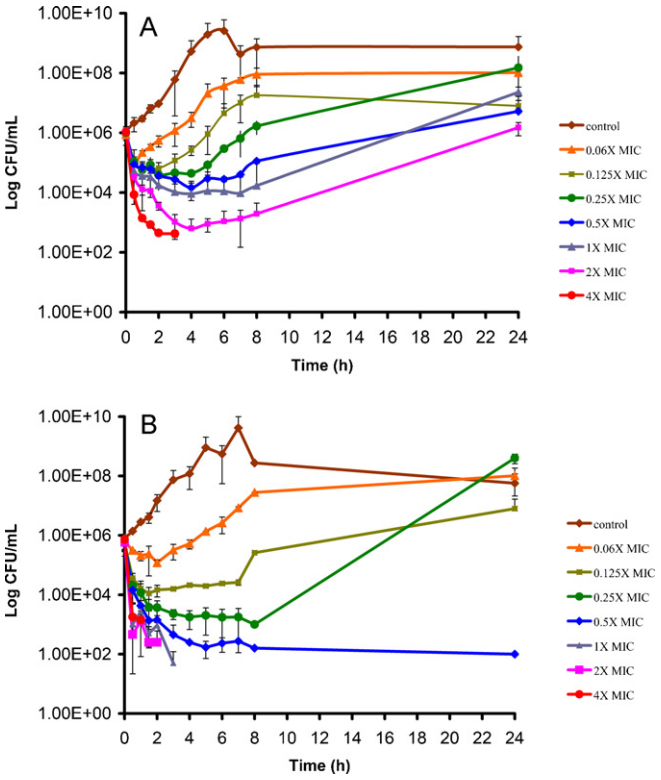

Fig. 4. Time-kill studies of MU1140 against *S. aureus*. (A) Time-kill studies in broth and (B) time-kill studies in 50% serum.

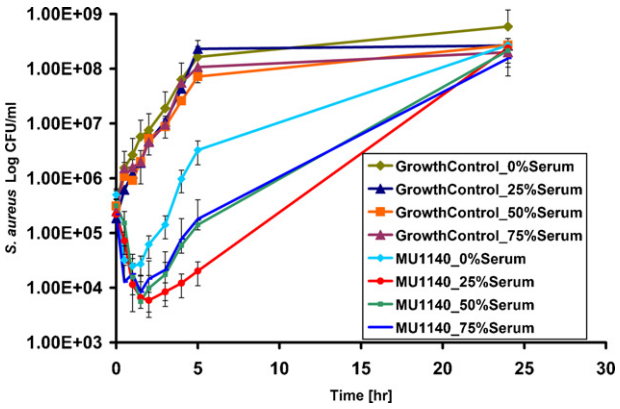

Fig. 6. Time-kill studies of MU1140 at 0.5 × MIC against *S. aureus* in the presence of various human serum concentrations (0%, 25%, 50%, and 75%).

ally, the rate of re-growth of the bacterial population also showed dependency on the serum content, where re-growth was fastest in the absence of serum, followed by 50% and 75% serum (which again were comparable), and the slowest rate of re-growth was observed in the presence of 25% serum. The growth controls for *S. aureus* in the media containing the different serum concentrations were comparable, suggesting that the observed differences could be ascribed to the interaction of MU1140 with serum and were not due to serum mediated inhibition of bacterial growth. Identical results were obtained when these studies was repeated using untreated serum (was not heat inactivated).

In an attempt to quantify the extent of augmentation of serum to MU1140 activity against *S. aureus*, mathematical modeling was used to estimate MU1140 specific parameters in the presence versus absence of serum. The model captured the observed data well. Curve fitting and the estimated model parameters are presented in Fig. 7 and Table 2, respectively. When compared to 0% serum, the calculated EC_{50} was 10- and 6-folds lower in the presence of 25% and 50% or 75% serum, respectively. Compared to neat broth, the elimination rate constant of MU1140 from the 25% and 75% serum-containing media was decreased 4-fold and 2.5-fold, respectively.

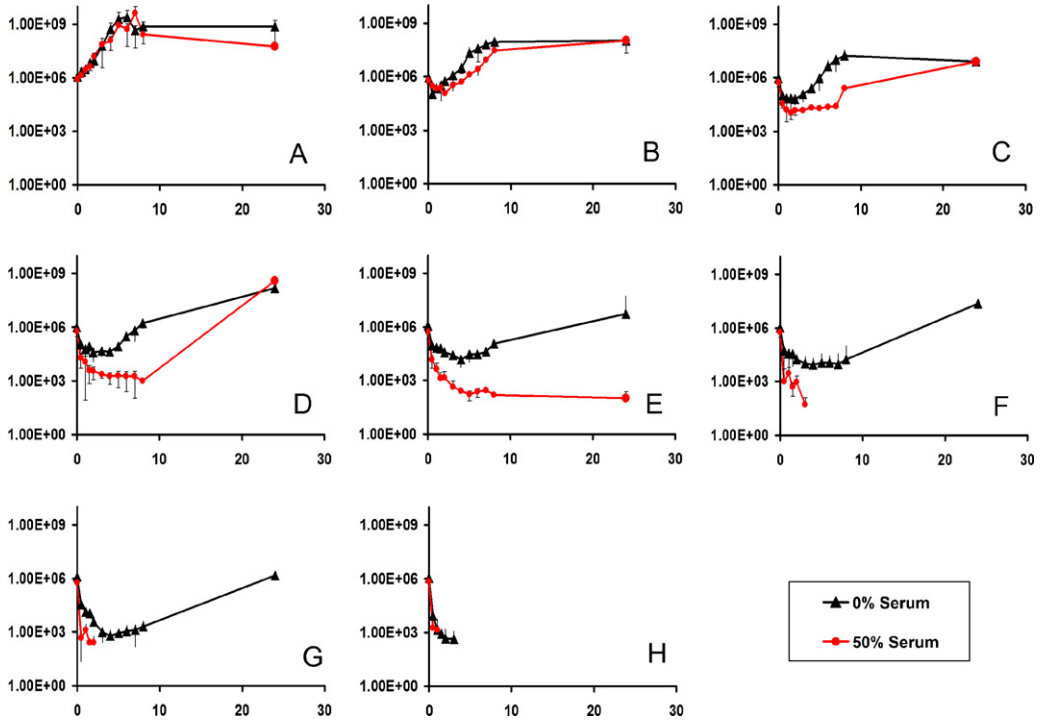

Fig. 5. Side-by-side *S. aureus* counts in flasks containing broth versus 50% serum and equivalent MU1140 concentrations. Log cfu/ml was plotted on the *y*-axis and time in hours was plotted on the *x*-axis. (A) Growth control, no MU1140. (B) 0.06 × MIC, (C) 0.12 × MIC, (D) 0.25 × MIC, (E) 0.5 × MIC, (F) 1 × MIC, (G) 2 × MIC, and (H) 4 × MIC.

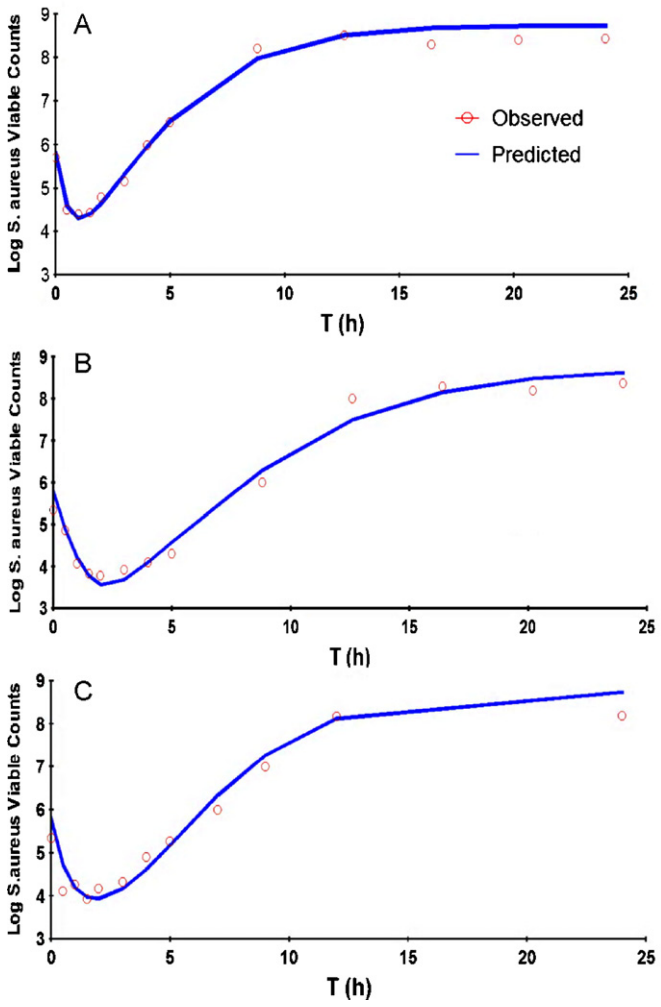

Fig. 7. Curve fits for MU1140 against *S. aureus* in the presence of (A) 0%, (B) 25%, and (C) 75% human serum. Symbols represent the observed cell counts and solid lines represent the curve fit based on the developed model.

Table 2
S. aureus PK/PD model parameter estimates.

Parameter	0% Serum	25% Serum	75% Serum
EC_{50} (μg/ml)	1×10^{-2}	1×10^{-3}	6×10^{-3}
k_e (h^{-1})	2.01	0.99	0.87

4. Discussion

It is generally believed that only the unbound fraction of an antibiotic is able to diffuse to the extravascular compartments where most bacterial infections are located (Cars, 1990; Kovar et al., 1997; Ogren and Cars, 1985; Scaglione et al., 1998), and exert its antibacterial activity. For highly bound drugs (>70%), binding to serum proteins is a crucial determinant of efficacy, since small changes in binding will produce large variations in the percentage of free and pharmacologically active drug (Craig and Kunin, 1976). This particular aspect is to be taken into account during dose selection and dosing regimen design for all drugs, especially for antibiotics since suboptimal antibiotic concentrations might result in therapy's failure (Chambers et al., 1984; Guttler et al., 1971; Calain et al., 1987) and is a risk factor for the development of antibiotic resistant strains (Schmidt et al., 2007).

To avoid the potential conflicts associated with media fortification with proteins (Schmidt et al., 2008), fresh human serum was used to evaluate the degree of MU1140 binding to serum proteins and the effect of serum on the bactericidal activity of MU1140. The data suggests that approximately 93% of MU1140 bound to serum components and the degree of binding was constant in the tested concentration range of 6.25–200 μg/ml. The pH of an MU1140 aqueous solution was determined to be 3.8 (data not shown), suggesting that, in serum, MU1140 would bind preferentially to albumin since acidic compounds typically show high binding affinity for the basic residues of albumin via ion-pairing (Ghuman et al., 2005). This hypothesis was not experimentally verified.

To predict the potential clinical efficacy of MU1140, the bactericidal activity of MU1140 in the presence of human serum was evaluated. The effect of the presence of serum proteins on the bactericidal activity of MU1140 was tested using two methods: (1) determination of MU1140 MIC against the strains of interest in the absence and the presence of various serum concentrations, and (2) by conducting time-kill studies of MU1140 against the bacterial strains in the absence and presence of serum. MIC determination showed that serum exerted a protective effect for *S. pneumoniae,* presumably by binding the antibiotic and reducing the active fraction, causing a rise in MU1140's MIC against *S. pneumoniae* from 0.8 μg/ml in the absence of serum to 1.6 and 3.2 μg/ml in the case of 25% and 50% medium serum content, respectively. The observed concentration-dependent inhibition by serum suggests that the pharmacodynamic activity of MU1140 was due to the free fraction of the antibiotic. It was assumed that the free fraction of MU1140 was proportional to the serum content of the media. Although this assumption was not validated experimentally, the linearity of binding of MU1140 to serum in the tested dose range is an indication that this assumption is valid. The increase of the MIC in serum of highly bound antibiotics is a phenomenon that has been observed both for small molecule antibiotics (Chambers et al., 1984; Jones and Barry, 1987; Lam et al., 1988) as well as for the peptides antibiotics LY333328 (Zhanel et al., 1998), daptomycin (Lee et al., 1991), and vancomycin (Dykhuizen et al., 1995). When the same experiment was repeated using *S. aureus*, the opposite outcome was observed where MU1140's MICs for *S. aureus* were decreased from 6.4 μg/ml in the absence of serum to 1.6 μg/ml in the presence of 25% or 50% serum. Previous studies has shown that MU1140 was stable in serum (Ghobrial et al., 2009a,b), thus the molecular basis for the effect of serum on the activity of MU1140 is unknown, and has not been reported for any lantibiotic.

Due to the inherit limitations of the MIC determination as an antibiotic activity characterization tool (Mueller et al., 2004), time-kill studies were performed as they provided a dynamic picture of the antibiotic's action at various concentrations throughout the study duration. In the first set of time-kill studies, the concentration of serum in the growth medium was maintained at 50% and the bacterial viable counts were determined in the presence of various MU1140 concentrations (0.25–16 × MIC). *S. pneumoniae* counts in serum were higher at all MU1140 concentrations when compared to the counts in broth. The inhibitory effect of the presence of serum on the activity of MU1140 was evident at concentrations below 4 × MIC. MU1140 activity seemed comparable in the presence and absence of serum at concentrations equivalent to and above 4 × MIC for *S. pneumoniae*. Presumably, under the experimental conditions, a threshold bactericidal concentration of free MU1140 in the presence of 50% serum was achieved at 4 × MIC. These results are in accord with the previously described MIC data. The opposite effect was observed in time-kill studies against *S. aureus* in the presence or absence of 50% serum. An obvious augmentation effect of serum was observed at MU1140 concentrations

above 0.06 × MIC. These results are in accord with the results of the MIC studies.

Time-kill studies of MU1140 against *S. aureus* in broth containing various amounts (25%, 50%, and 75%) of serum showed that *S. aureus* cell counts were lowest in flasks containing 25% serum while the bacterial numbers were equivalent in the 50% and 75% serum flasks. The interpretation of these results is not very obvious, but the data suggests that a complex process is in place where serum components synergistically augmented MU1140 killing of *S. aureus*, but at the same time, at low serum concentrations, not drastically lowering the free MU1140 fraction and, hence, maximum kill was observed at 25% serum content. Hypothetically, this augmentation could be due to the interaction of serum components with specific extracelluar sites on *S. aureus*, thus acting as a docking site for MU1140 and facilitating the MU1140–lipid II interaction.

PK/PD modeling was utilized to quantify the augmentative effect of serum on MU1140 activity against *S. aureus*. The assumption of the model is that binding of MU1140 to the bacterial cell wall components is irreversible, and thus the antibiotic concentration is not static in the growth media, but rather in continuous decrease as the bacteria is multiplying. This was modeled as an elimination process. This hypothesis was based on earlier work (Smith and Hillman, 2008; Smith et al., 2008) and on the observation that the concentrations of free MU1140 in the time-kill study growth media (without serum) were below the limits of detection shortly after antibiotic addition (data not shown). It was also observed that the MIC of MU1140 for the *S. aureus* culture before and after the time-kill study was not altered (data not shown); hence a resistant subpopulation cannot be implicated in the observed re-growth phenomenon.

The PK/PD model parameter estimates included the potency of MU1140 (EC_{50}) and its elimination rate constant (k_e). In accordance with the observed cell counts, MU1140 EC_{50} was lowest when human serum was present at 25%, followed by 50% or 75%, and the highest EC_{50} was predicted in the absence of serum. The model predicted MU1140 elimination rate constant (k_e) due to binding to bacterial lipid II, was lowest when serum was at its highest concentration and the highest rate of elimination was observed at 0% serum. This further suggests that serum is acting as a reservoir for MU1140 is in competition with the bacterial cells for this antibiotic; hence the higher serum levels conferred more protection for *S. aureus* than the lower serum concentrations. In addition, this explains the observed serum concentration-dependent re-growth rates of *S. aureus*.

The data presented suggests that MU1140 is potentially active *in vivo* against the tested strains, at least in the central compartment. The observed behavior against *S. aureus* suggests the presence of a complex relationship of augmentative and inhibitory interactions between this lantibiotic, human serum, and *S. aureus* that is worthy of further investigation.

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