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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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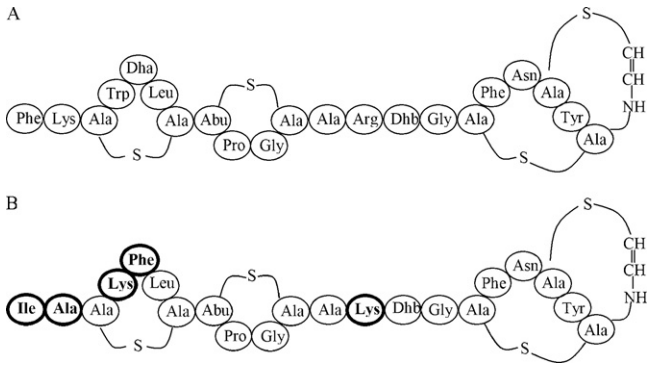

Fig. 1. MU1140 and gallidermin, amino acids specific to gallidermin are highlighted (A) MU1140 (Hillman et al., 1998) and (B) gallidermin.

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).