Effect of Serum and Albumin on MIC and MBC of Daptomycin and Comparator Agent for 20 S. aureus and 10 Enterococci

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Abstract

Background: The addition of serum and albumin (ALB) to testing media, will affect the free calcium concentration ([Ca++]f) which may impact in vitro activity. This study examined the in vitro susceptibility of 20 daptomycin (DAP) and 10 comparator agent (COMP) strains with and without serum (SE) or ALB with free calcium adjusted with or without serum (SE) or ALB with free calcium adjusted with or without serum (SE) or ALB. Methods: Daptomycin MIC and MBC (CLSI broth microdilution) for 20 clinical isolates (10 S. aureus, 10 enterococci) were determined in broth supplemented with 50 µg/mL Ca++. CSMHB, COMP: 50% human serum adjusted to 50 µg/mL Ca++. Vancomycin was also analyzed using the same method. Geometric means (GM) were used for results. Results: Geometric Mean MICs and MBCs were similar to MICs when ALB is added, regardless of calcium adjustment. MBCs tend to be 1-2 dilutions higher than MICs. When Ca++ in the CSMHB (no ALB) was adjusted to 50 µg/mL, 9 S. aureus and 4 enterococci strains had increased MICs and MBCs due to reversible binding of daptomycin to serum albumin. Conclusions: Results indicate that in vitro susceptibility testing should be performed with ALB adjusted to physiological levels of free calcium ([Ca++]f) and serum albumin (ALB) and used in the methods for reporting and interpretation of the results. Additional studies of the interaction of MICs and MBCs with serum and ALB is warranted.

Introduction

Various in vitro studies have shown the effect of addition of serum and albumin on daptomycin’s bactericidal activity [1]. Since free calcium ions (Ca++) will bind to serum proteins and albumin, additional calcium is required in order to simulate physiological levels. This study was performed to provide more in depth MIC and MBC data (compared to prior studies) with and without serum (50% human serum adjusted to 4 g/dL ALB) with 20 S. aureus and 10 enterococci.

Materials & Methods

Antimicrobial Agents Daptomycin: Ciba-Geigy (Fara, NJ), lot 9850554-1, 62.3 mg/L. Vancomycin: Ciba-Geigy (Fara, NJ), Lot 6080131, Concentration: 5.25–256 µg/mL.

Microorganisms


Methods

Physiological levels of ionized calcium in serum (approximately 50 µg/mL) are observed if a blood sample is collected in a glass or plastic (EDTA) vial. In whole blood, ionized calcium may increase by as much as a 10% increase. To avoid this, serum was collected in a glass vial. Prior to analysis, the ionized calcium was adjusted to a level considered to be ‘physiological’. This was done by adding a known amount of calcium chloride to each test tube and adjusting the concentration of ionized calcium in serum. An additional study was performed with a lower concentration of ionized calcium (25 µg/mL) to determine the effect of a lower concentration of ionized calcium. All studies included serum and ALB. The ALB was added to the broth to simulate physiological levels of ionized calcium in serum and to maintain the appropriate level of free calcium in the broth. In this study, the ALB was added after the susceptible organisms were added and mixed in the broth to avoid the effects of free calcium on the free calcium in the broth. The added calcium chloride was necessary to achieve a 50 µg/mL level of free calcium. Numerous studies have shown that the addition of calcium chloride in broth can increase the free calcium in the broth [2-3]. MBCs were determined according to CLSI broth microdilution and bactericidal activity determination procedures [4]. Microorganisms were added to each test tube in 250 µL of broth to obtain an inoculum of approximately 2-5 x 10^7 CFU/mL. A fresh stock of bacteria was used for each test. Protein binding for daptomycin was determined by an ELISA [4] and for vancomycin was determined by an ELISA [5]. MIC and MBC results were determined according to CLSI broth microdilution and bactericidal activity determination procedures [4]. The MBC and MBC/B MIC ratio were calculated as day 1, and day 2 for S. aureus. The MIC was determined by CLSI broth microdilution and the MBC was determined by the broth microdilution with a 24 hour incubation period. The MBC/B MIC ratio was calculated as the MBC divided by the MIC.

Results

Daptomycin MICs and MBCs were similar to MICs when ALB is added, regardless of calcium adjustment. MBCs tend to be 1-2 dilutions higher than MICs. When Ca++ in the CSMHB (no ALB) was adjusted to 50 µg/mL, 9 S. aureus and 4 enterococci strains had increased MICs and MBCs due to reversible binding of daptomycin to serum albumin (SA).

Conclusions

The effects of daptomycin and vancomycin on bacterial MICs and MBCs were investigated using human serum and albumin. In addition, the addition of serum and albumin can change free calcium ion levels, they were maintained in this study at approximately physiological levels (50 µg/mL). While MICs of vancomycin were not influenced by addition of serum, albumin or calcium ion concentration, the MBCs of daptomycin for both S. aureus and enterococci were elevated. Although the MICs and MBCs increased with addition of serum or albumin, the MBC/MIC ratio for daptomycin remained similar to the ratio with no serum or albumin added.

References


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