Introduction

Quinupristin/dalfopristin, a new semisynthetic parenterally administered streptogramin antibiotic, is a 30:70 mixture of two water-soluble compounds, quinupristin and dalfopristin, derived from natural pristinamycins I and II, respectively. Streptogramin antibiotics are similar in their mechanism of action to macrolides and lincosamides in that they inhibit protein synthesis in bacteria by affecting ribosome function. The proposed mechanism of action of quinupristin/dalfopristin is that protein synthesis is inhibited by conformational modification of the region of the bacterial ribosome where peptidyltransferase acts. This in turn may decrease the hydrolytic activity of peptidyl-tRNA hydrolase leading to a tRNA 'auxotrophy' and bacterial cell death.

A previous study of the in-vitro activity of quinupristin/dalfopristin demonstrated that 17 of 50 Staphylococcus aureus strains tested had MBCs at least four-fold higher than their MICs and some strains had an MBC up to 16-fold higher. Six strains are known to be resistant to macrolide, lincosamide and streptogramin B type (MLS)

Some strains of staphylococci have raised MBCs of quinupristin/dalfopristin compared with their MICs. In this study, the time–kill kinetics of quinupristin/dalfopristin at 2 mg/L on two strains of Staphylococcus aureus were determined by viable count and intracellular ATP measurement. After 24 h exposure to quinupristin/dalfopristin, the percentage survival of the strain with a raised MBC was 5.9 and that of the strain with a normal MBC was 0.04. The time–kill kinetics of the strain with a raised MBC were analogous to those associated with phenotypic tolerance.

Materials and methods

The clinical isolates of S. aureus studied were F31 (normal MBC, MLS

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to reach logarithmic growth phase. For the stationary phase inocula the above dilutions were performed just before antibiotic addition. A stock solution of quinupristin/dalfopristin (1000 mg/L) was prepared in sterile distilled water, filter sterilized and added to the cultures to give a final concentration of 2 mg/L. For each organism, antibiotic-free controls were included for each growth phase and inoculum. The cultures were placed in a shaking water bath at 35–37°C and incubated for the duration of the study.

Viable counting

Viable counts were determined on 5% horse blood agar plates at 0, 4, 8 and 24 h after appropriate dilution in phosphate-buffered saline pH 7.3 (Unipath) with a spiral plater (Don Whitley, Skipton, U K) and after 48 h incubation at 35–37°C the colonies were counted. Means of three replica viable counts (cfu/mL) were plotted logarithmically against time.

Intracellular ATP determination

Intracellular ATP was determined at 0, 4, 8 and 24 h. The culture samples were incubated with 0.04% apyrase grade I (Sigma Chemical Co. Ltd, Poole, U K) for 10 min at 37°C to remove any extracellular ATP present. ATP was extracted from the bacterial samples by incubation at room temperature for 10 min with an equal volume of 2% trichloroacetic acid (TCA) (BDH Merck Ltd, Poole, U K) containing 0.04 M ethylenediaminetetraacetic acid (EDTA) (BDH Merck Ltd) which inhibits ATP-hydrolysing enzymes. The samples were then diluted with Tris-acetate buffer and 2 mM EDTA (Bio Orbit, Turku, Finland) and an aliquot was then assayed with firefly luciferin–luciferase ATP monitoring reagent (Bio Orbit) on a 1251 luminometer (Bio Orbit). ATP concentrations of samples were calculated based on the luminescence produced and calibrated by an internal ATP standard. The mean of two replica ATP values (μmol/mL) were plotted logarithmically against time.

Results

Figures 1 and 2 illustrate the time–kill kinetics of 2 mg/L quinupristin/dalfopristin on S. aureus strains F31 (normal MBC) and F457 (raised MBC), respectively, in log phase evaluated by viable counts and intracellular ATP determinations.

The ATP values throughout exhibited good correlation with viable counts for the unexposed control cultures, although less correlation was observed between ATP
values and viable counts in the antibiotic-exposed cultures.

The log phase cultures of both strains were more susceptible to quinupristin/dalfopristin than the stationary phase cultures, as shown by the bactericidal rates (change in $\log_{10}$ cfu/mL from 0 to 4 h) (Table). The bactericidal rates were $-1.62$ and $-1.41$ for log phase cultures and $-0.58$ and $0.05$ for stationary phase cultures, at inocula of $10^6$ cfu/mL of F31 and F457, respectively. Quinupristin/dalfopristin exhibited more bactericidal activity against the $10^6$ cfu/mL inoculum than the $10^8$ cfu/mL inoculum in both growth phases (Table).

As expected, quinupristin/dalfopristin at 2 mg/L exhibited more bactericidal activity on *S. aureus* strain F31 than on strain F457.

For log phase cultures with initial inocula of $10^6$ cfu/mL, the two *S. aureus* strains exhibited different reductions in viable counts over the test period.

**Discussion**

Time–kill kinetic techniques demonstrated a better correlation with in-vivo efficacy than other methods of determining bactericidal activity; time–kill kinetic techniques are also the most reliable method for determining and differentiating tolerance. Conventional viable counts allow morphological and culture purity checks to be performed but the method is retrospective, with cfu/mL calculated after 48 h incubation. Intracellular ATP measurement is rapid, data being available 30 min after sampling, and may allow determination of viable but non-culturable organisms, that is organisms that are still synthesizing RNA and protein. The reduced correlation observed between viable counts and intracellular ATP measurement in antibiotic-exposed cultures might be due to viable but non-culturable organisms and might reflect the time difference in data collection between the two methods.

It has been shown that phenotypically tolerant isolates demonstrate time–kill kinetics that exhibit an initial high rate of killing similar to a non-tolerant isolate but with a greater survival rate (>$0.1\%$), whereas genetically tolerant isolates demonstrate a slow loss of viability over the entire killing curve. The killing curve of log phase F457 with an initial inoculum of $10^6$ cfu/mL demonstrated an initially high decrease in viability of $1.4 \log_{10}$ cfu/mL at 4 h, similar to that of F31 ($1.6 \log_{10}$ cfu/mL) at 4 h in the same conditions. F457 had a higher survival rate ($5.9\%$ after 24 h), that is the reduction in viability at 24 h was $1.2 \log_{10}$ compared with a reduction in viability at 24 h of $3.4 \log_{10}$ for F31 ($0.04\%$ survival rate after 24 h). From these results it could be speculated that the raised MBC of quinupristin/dalfopristin for F457 is due to phenotypic tolerance, defined as decreased susceptibility to antimicrobials under certain growth conditions. We have previously compared the post-antibiotic effect of quinupristin/dalfopristin on *S. aureus* strains with and without raised MBCs; no significant differences were observed between the two populations.

The clinical significance and the mechanism of tolerance of *S. aureus* strains that have raised MBCs of quinupristin/dalfopristin has yet to be determined. Investigators have demonstrated that some constitutive MLS$_S$-resistant *S. aureus* also have raised MBCs and diminished killing effects in time–kill experiments with quinupristin/dalfopristin. It has been proposed that clinical treatment with quinupristin/dalfopristin of infections caused by MLS$_S$-resistant *S. aureus* that require bactericidal activity (e.g. endocarditis) might be successful only against strains with a low quinupristin/dalfopristin MBC. Carefully conducted clinical studies are required to obtain answers to these important questions.

**Acknowledgements**

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**References**


**Table. Bactericidal rates (change in $\log_{10}$ cfu/mL from 0 to 4 h) of 2 mg/L quinupristin/dalfopristin**

<table>
<thead>
<tr>
<th>Strain</th>
<th>log phase</th>
<th>$10^6$ cfu/mL</th>
<th>Stationary phase</th>
<th>log phase</th>
<th>$10^8$ cfu/mL</th>
<th>Stationary phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>F31</td>
<td>$-0.69$</td>
<td>$-0.36$</td>
<td>$-1.62$</td>
<td>$-0.58$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F457$^a$</td>
<td>$-0.3$</td>
<td>$-0.1$</td>
<td>$-1.41$</td>
<td>$0.05$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Associated with raised MBC.


