Scanning electronmicroscopy of *Staphylococcus aureus* and *Enterococcus faecalis* exposed to daptomycin

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Summary. The novel lipopeptide antibiotic, daptomycin, at a concentration of 8 mg/L, caused gross morphological changes in both a methicillin-sensitive and a methicillin-resistant strain of *Staphylococcus aureus* and in a strain of *Enterococcus faecalis*. The earliest (after 1 h) surface lesion observed was the appearance of boss-like processes randomly distributed on the cell surface. Later, grossly deformed bacteria were seen and in two of the three bacteria prolonged exposure led to degeneration of the cells into an amorphous syncytial mass. Omission of calcium (which is known to potentiate the activity of daptomycin) from the culture medium did not affect the morphological response to an inhibitory concentration of the antibiotic.

Introduction

Daptomycin (formerly known as LY146032) is a novel lipopeptide related to the antibiotic complex A21978C of *Streptomyces roseosporus*. The activity of daptomycin is restricted to aerobic and anaerobic gram-positive cocci (Eliopoulos *et al.*, 1986; Ehlert and Neu, 1987; Greenwood and Palfreyman, 1987) and requires the presence of calcium for its full expression (Eliopoulos *et al.*, 1986; Andrew *et al.*, 1987).

The mechanism of action of daptomycin has not been completely elucidated. Present evidence favours the view that the antibiotic interferes with an early stage in the biosynthesis of peptidoglycan, but the compound also affects the integrity of the cell membrane (Lakey and Lea, 1986; Allen *et al.*, 1987). The ability of calcium to potentiate the activity of daptomycin is probably related to a selectively increased penetration of the lipid moiety of the lipopeptide into the phospholipid of the cell membrane (Lakey and Ptak, 1988).

Scanning electronmicroscopy offers the unique ability to examine surface structures at relatively high resolution and has proved particularly useful in the examination of the effect of antibiotics that act on the bacterial cell wall (Greenwood and O’Grady, 1969, 1972; Elliott and Greenwood, 1983). In the present study the technique has been used to examine the effects of daptomycin on selected gram-positive cocci in the presence and absence of calcium.

Materials and methods

Bacteria

Two strains of *Staphylococcus aureus* (one methicillin-sensitive and one methicillin-resistant) and a strain of *Enterococcus faecalis* were randomly selected from clinical isolates used in a previous study (Andrew *et al.*, 1987).

Culture medium

Iso-Sensitest Broth (Oxoid) was prepared in deionised water. For supplementation with calcium, analytical grade CaCl₂·2H₂O was prepared in deionised water, sterilised by filtration and added to sterilised broth to achieve a concentration of 2·5 mM.

Antibiotic

Daptomycin was supplied by Lilly Research, Windlesham, Surrey. Solutions of antibiotic were freshly prepared in sterile deionised water. For supplementation with calcium, solutions were prepared and further diluted in broth.

Exposure to antibiotic

Cultures inoculated into Iso-Sensitest broth were incubated at 37°C in the light path of a continuous opacity monitoring device (Watson *et al.*, 1969). Daptomycin was added at a standard point in the exponential

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phase of growth at which the bacterial population was \( c. 10^8 \) cfu/ml.

Inhibitory activity of daptomycin was estimated by turbidimetric experiments in which bacteria were exposed to a range of antibiotic concentrations. For morphological studies, concentrations were chosen that caused complete inhibition of growth as judged turbidimetrically.

**Scanning electronmicroscopy**

Samples were removed before, and at intervals after, exposure to daptomycin and processed for scanning electronmicroscopy as described by Elliott and Greenwood (1983). Briefly, the samples were fixed for 1 h (glutaraldehyde 5% in 0.05 M sodium cacodylate containing 10 mM MgSO\(_4\)), washed by centrifugation and dehydrated in alcohol, followed by acetone. The bacterial suspension was then critical-point dried, coated with gold and examined in a Jeol 100C electronmicroscope.

**Results**

**Turbidimetric studies**

The in-vitro activity of daptomycin against the test strains, as judged turbidimetrically, is shown in the table. In the presence of a physiological concentration of calcium (2.5 mM), daptomycin 0.12 mg/L caused partial inhibition of growth, but a concentration of 1–2 mg/L was required to prevent growth during overnight incubation. In the absence of calcium, much higher concentrations of daptomycin were required to achieve comparable degrees of growth suppression. In experiments in which dense bacterial populations (c. \( 10^8 \) cfu/ml) were exposed to daptomycin in the presence of 2.5 mM calcium, concentrations of daptomycin exceeding 8 mg/L caused no further change in the turbidimetric record with any of the three strains and for this reason this concentration was chosen for the morphological studies. The turbidimetric response of dense populations of the three test organisms to daptomycin 8 mg/L is shown in fig. 1. The marked fall in opacity observed following addition of the antibiotic to cultures of the methicillin-resistant strain of *S. aureus* and the strain of *E. faecalis* was less marked in the case of the methicillin-sensitive strain of *S. aureus*.

**Morphological studies**

The appearance of the methicillin-sensitive strain of *S. aureus* in the absence of antibiotics is shown in fig. 2A. The majority of the bacteria were smooth and rounded, but not always spherical. The

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Inhibitory concentration (mg/L)</th>
<th>MAC</th>
<th>MIC</th>
<th>MAC</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> (methicillin-sensitive)</td>
<td>in presence of Ca(^{++})</td>
<td>0.12</td>
<td>1</td>
<td>64</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>S. aureus</em> (methicillin-resistant)</td>
<td>in absence of Ca(^{++})</td>
<td>0.12</td>
<td>1</td>
<td>64</td>
<td>&gt;64</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td></td>
<td>0.12</td>
<td>2</td>
<td>128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

MAC = minimum antibacterial concentration (concentration causing a deviation from normal growth); MIC = minimum inhibitory concentration (concentration causing suppression of growth during overnight incubation).

**Table. Inhibitory activity of daptomycin for the test strains as judged turbidimetrically**

**Fig. 1. Continuous opacity records of**

(A) *S. aureus* (methicillin-sensitive); (B) *S. aureus* (methicillin-resistant); (C) *E. faecalis*. Daptomycin was added at arrow to achieve a concentration of 8 mg/L.
organisms were grouped in characteristic grape-like clusters. After exposure to daptomycin 8 mg/L for 1 h, the surface of the bacteria appeared roughened with occasional boss-like protuberances, and after 4 h most of the bacteria exhibited these processes (fig. 2B). After exposure to daptomycin for 24 h affected cells exhibited grossly altered surfaces and very prominent cleavage sites (fig. 2C).

The effects observed with the methicillin-resistant strain of *S. aureus* were somewhat different: unexposed cells commonly displayed small surface projections (fig. 3A); cells exposed to daptomycin for 1 or 4 h (fig. 3B) exhibited bizarre defects that appeared to be particularly associated with cleavage sites; and overnight exposure reduced the bacteria to an amorphous syncytium (fig. 3C).

The characteristic elongated diplococci of *E. faecalis* are shown in fig. 4A. After exposure to daptomycin for 1 h a few cells exhibited roughened surfaces with occasional extrusions that were commonly associated with incipient sites of division, or with the poles of the cell; exposure for 4 h did not appear to lead to further gross alterations, but after overnight incubation the bacteria were barely recognisable as streptococci (fig. 4B).

The methicillin-sensitive strain of *S. aureus* was also examined for its response to daptomycin in the absence of calcium. In these experiments the bacteria were exposed to daptomycin 64 mg/L, which caused a similar turbidimetric response to that obtained with cultures exposed to 8 mg/L in the presence of calcium. The morphological effects observed were very similar to those found in the presence of calcium: small, randomly distributed lesions were visible after exposure for 1–4 h with severe disruption of the cell wall on prolonged exposure to daptomycin.

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**Fig. 2.** Scanning electronmicrographs of *S. aureus* (methicillin-sensitive) exposed to (A) no antibiotic; (B) daptomycin 8 mg/L for 4 h; (C) daptomycin 8 mg/L for 24 h. Bar = 1 μm.
Discussion

These results clearly demonstrate that daptomycin causes gross morphological alterations to the surface of gram-positive cocci. The results are consistent with the postulated mechanism of action of the antibiotic in interfering with the biosynthesis of peptidoglycan (Allen et al., 1987).

The earliest change induced by daptomycin appears to be the production of small surface projections not unlike those seen in staphylococci exposed to penicillins (Greenwood and O'Grady, 1969). However, the gross abnormalities observed in the methicillin-sensitive strain of *S. aureus* after prolonged exposure (fig. 2C) and in the methicillin-resistant strain in the earlier stages of drug exposure (fig. 3B) are more suggestive of the continued production of defective cell-wall material, and continued, but aberrant septation.

Overnight exposure to daptomycin of the methicillin-resistant strain of *S. aureus* and the strain of *E. faecalis* caused total distortion of the bacterial surface so that the cells were reduced to a formless mass. In contrast, grossly abnormal, but recognisable cells remained after overnight exposure of the methicillin-sensitive strains to daptomycin. Such strain variation in response to daptomycin may explain differences in the rate of killing of staphylococci and enterococci reported by various authors (Ehlert and Neu, 1987; Jorgensen et al., 1987; Machka and Braveny, 1987; Verbist, 1987).

Omission of calcium from the culture medium did not appear to affect the morphological response to daptomycin, so that the morphological consequences of the antibiotic's activity are evidently not dependent on the cation. These results, therefore, support earlier speculation that calcium aids intracellular penetration of the antibiotic to its target site (Lakey and Lea, 1986; Allen et al., 1987; Lakey and Ptak, 1988).

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Fig. 4. Scanning electronmicrographs of *E. faecalis* exposed to (A) no antibiotic; (B) daptomycin 8 mg/L for 24 h. Bar = 1 μm.

REFERENCES


