Reversing methicillin resistance in MRSA using a bacterial transforming agent

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Background: Antimicrobial resistance in staphylococci remains a significant problem in the clinical management of infections. New therapeutic entities are required for the prophylaxis and treatment of staphylococcal infection including those caused by methicillin-resistant Staphylococcus aureus (MRSA). Potential candidates include bacterial transforming agents (BTAs), compounds that can potentiate the activity of cell-wall-active antimicrobials by hypersensitizing the bacterial cell wall to the bactericidal effects of these drugs. BTAs have been found to inhibit MRSA in vitro when administered in combination with established antibiotics.

Objectives: To examine the antimicrobial potential of a known BTA (BTA 19976a) on strains of MRSA in vitro.

Methods: Etest and time–kill methodologies were employed to assess the inhibitory potential of BTA at 10% w/v on strains of E-MRSA-3, E-MRSA-15 and E-MRSA-16.

Results: Etests demonstrated a reduction in the oxacillin MIC for E-MRSA-3, E-MRSA-15 and the NCTC 12493 reference strain of MRSA when exposed to BTA at 10% w/v. Time–kill assays similarly demonstrated a reduction in viable counts for organisms exposed to methicillin at 40 mg/L + BTA at 10% w/v, compared with methicillin alone, an effect which varied in cidality, pattern of killing and regrowth between strains.

Conclusions: The antimicrobial effects of this BTA on MRSA are encouraging and warrant further investigation with large numbers of different epidemic strains and a comprehensive PK/PD evaluation. This could lead to new therapeutic entities for the prophylaxis and treatment of staphylococcal infections.

Keywords: anti-infective development, drug susceptibility testing, β-lactams, cell wall, hypersensitizing agent

Introduction

Staphylococcal resistance to antibiotics remains an increasing problem in the prophylaxis and treatment of staphylococcal infections. Compounds have been described, which act by hypersensitizing the bacterial cell wall and reversing in vitro resistance to β-lactam antibiotics. They have been termed ‘bacterial transforming agents’ (BTAs) by their advocates.1

The transforming effect of these compounds refers to the rendering of previously non-susceptible bacteria to a state of susceptibility to cell-wall-active antibiotics rather than any reference to uptake of DNA.

BTAs do not interfere with specific bacterial resistance mechanisms on their own, but rather potentiate the activity of antimicrobial agents that act on the cell wall by targeting moieties involved in the binding of muropeptide tails to cell-wall cross links.

Previous experiments have shown that high concentrations of glycine in culture medium will affect staphylococcal cell wall synthesis.2 Bacterial cell wall peptidoglycan muropeptides with a D-Ala-D-Ala terminus are replaced with a D-Ala-glycine terminus, resulting in a concomitant decrease in methicillin resistance. This appears to indicate a central role for D-Ala-D-Ala-terminating precursors as substrates for efficient peptidoglycan synthesis by PBP2a in Staphylococcus aureus.

BTAs are thought to alter the terminating muropeptide tail from D-Ala-D-Ala to D-Ala-BTA, and the inability of PBP2a to use this substrate for peptidoglycan synthesis confers the observed decrease in resistance to methicillin.1

We sought to evaluate one particular BTA molecule currently under patent (BTA 19976a) to examine its in vitro activity against S. aureus using Etest and time–kill methodologies.

Materials and methods

One strain each of E-MRSA-3, E-MRSA-15 and E-MRSA-16 were kindly provided by the Scottish MRSA Reference Laboratory. We
included the methicillin-resistant *S. aureus* (MRSA) strain NCTC 12493. Methicillin susceptibility powder was obtained from USP Ltd (Maryland, USA), BTA from Sigma (Dorset, UK) and oxacillin Etests from AB Biodisk (Solna, Sweden). Etest susceptibility testing utilized both Iso-Sensitest agar (ISA) and Mueller–Hinton agar (MHA) (Oxoid, UK), and Etests were performed according to manufacturers’ instructions and current guidelines.\(^3\) BTA powder was incorporated into the agar to achieve a final concentration of 10% w/v. Tests were performed in duplicate and MICs calculated for each isolate.

Time–kill experiments were performed according to CLSI guidelines using cation-adjusted Mueller–Hinton broth (Oxoid UK).\(^4\) A concentration of 40 mg/L methicillin was chosen to reflect a comparable serum concentration obtainable *in vivo* immediately after a standard 2 g intravenous dose of flucloxacillin.\(^5\) Organisms were exposed to methicillin at a concentration of 40 mg/L and to methicillin 40 mg/L plus BTA 10% w/v. Tests were carried out in duplicate with growth and sterility controls included on each occasion. An initial inoculum of between \(5 \times 10^5\) and \(5 \times 10^6\) cfu/mL was deemed acceptable when determined from the untreated growth control. Viable colony counts were determined at time = 0, 4, 8, 24 and 48 h, by serial dilution, inoculation onto Columbia agar and incubation for 24 h. An average viable colony count was calculated for each time interval from the time–kill analyses.

### Results

Results of oxacillin MICs by Etest are presented in Table 1. BTA incorporated at 10% w/v in the medium caused a reduction in the oxacillin MICs for all isolates on ISA and for all except E-MRSA-16 on MHA + 2% NaCl. E-MRSA-3, E-MRSA-15 and NCTC 12493 strains were rendered ‘susceptible’ when exposed to BTA on ISA. Notably, the MICs determined on MHA + 2% NaCl with BTA 10% w/v are higher than for ISA, and no transforming effect is seen for E-MRSA-16. This suggests that different culture media have an effect upon the transforming potential of BTA molecules and warrants further investigation.

Time–kill assays were analysed using the viable colony count method. A bactericidal effect was defined as a decrease of \(3 \log_{10}\) cfu/mL in the original inoculum, and a bacteriostatic effect if reduced by 0 to \(<3 \log_{10}\) cfu/mL. Results of time–kill analysis for each strain of MRSA are shown in Table 1 and Figure 1. There was a reduction in viable counts for organisms exposed to methicillin at 40 mg/L + BTA at 10% w/v compared with methicillin alone, an effect that varied in cidality, pattern of killing and regrowth between strains. E-MRSA-16 was more resilient to the transforming effects of BTA compared with, for example, E-MRSA-3, showing only a marginal bacteriostatic effect at 24 h and regrowth at 48 h.

### Discussion

The anti-infective technology of BTAs has recently been described.\(^1\) *In vitro* results with this particular BTA show promise for the use of such compounds in new therapeutic strategies for decolonization or prophylaxis for prosthetic devices, in order to increase the susceptibility of potential pathogens to antibiotics *in vivo*. Other BTA compounds may harbour transforming activity at lower concentrations and might be useful as possible co-formulants with \(\beta\)-lactam antibiotics. A previous study has shown the inferiority of glycopeptide therapy compared with \(\beta\)-lactam therapy against methicillin-susceptible *S. aureus* clinical infections.\(^6\) The possibility of new therapies, which would restore activity of traditional \(\beta\)-lactam agents against MRSA, is an exciting prospect, given the relative paucity of new drugs for these multiresistant organisms.

The clinical use of any BTA would be dependent on not only its antimicrobial properties when combined with a \(\beta\)-lactam antibiotic but also evidence that it would not induce glycopeptide

Table 1. Etest and time–kill analyses for MRSA strains against methicillin with and without BTA 19976a

<table>
<thead>
<tr>
<th>NCTC 12493</th>
<th>E-MRSA-3</th>
<th>E-MRSA-15</th>
<th>E-MRSA-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin MIC (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISA control</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>ISA + BTA 10% w/v</td>
<td>0.125</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MHB + 2% NaCl control</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>MHB + 2% NaCl + BTA 10% w/v</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Inhibitory effect (log\(_{10}\) cfu/mL change in viable colony count)

<table>
<thead>
<tr>
<th>Time–kill analysis</th>
<th>NCTC 12493</th>
<th>E-MRSA-3</th>
<th>E-MRSA-15</th>
<th>E-MRSA-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>methicillin 40 mg/L at 24 h</td>
<td>indifference (+0.3)</td>
<td>none (≥2)</td>
<td>none (≥2)</td>
<td>none (≥2)</td>
</tr>
<tr>
<td>methicillin 40 mg/L + BTA 10% w/v at 24 h</td>
<td>bacteriostatic (–1.8)</td>
<td>bactericidal (–3.6)</td>
<td>bactericidal (–3.1)</td>
<td>bacteriostatic (–1.2)</td>
</tr>
<tr>
<td>methicillin 40 mg/L at 48 h</td>
<td>none (≥2)</td>
<td>none (≥2)</td>
<td>none (≥2)</td>
<td>none (≥2)</td>
</tr>
<tr>
<td>methicillin 40 mg/L + BTA 10% w/v at 48 h</td>
<td>bactericidal (–3.5)</td>
<td>bactericidal (–3.6)</td>
<td>indifference (+0.3)</td>
<td>none (≥2)</td>
</tr>
</tbody>
</table>
Reversing methicillin resistance in MRSA

Figure 1. Time–kill analyses for MRSA strains against methicillin with and without BTA 19976a. Filled squares, untreated growth control; open diamonds, methicillin 40 mg/L; filled triangles, methicillin 40 mg/L + BTA 10% w/v; open circles in the graph for E-15 represent BTA 10% w/v only, showing lack of transforming activity without methicillin after 8 h of incubation. Note the differences in kill curves between strains; BTA prevents regrowth of NCTC and E-MRSA-3 strains after 24 h of incubation, but not of E-MRSA-15 and E-MRSA-16.

Transparency declarations
None to declare.

References

Acknowledgements
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resistance as a result of its interactions with bacterial peptidoglycan muropeptides.

In fact, BTAs have previously been shown to hypersensitize the bacterial cell wall to the antimicrobial activity of vancomycin with a reduction in the MIC, including that for MRSA with reduced glycopeptide susceptibility and for vancomycin-resistant Enterococcus faecium. BTA 19976a warrants a comprehensive in vitro assessment at different concentrations regarding its effects on vancomycin susceptibility in S. aureus.

BTAs may offer new therapeutic options in the treatment of staphylococcal and other bacterial infections by co-formulation with cell-wall-active antibiotics.

In vitro analysis of BTA 19976a using Etest and time–kill methodologies shows good antimicrobial potential for this compound against S. aureus. The inhibitory ability of this and other BTA molecules in combination with cell-wall-active antibiotics deserves further evaluation with a range of MRSA and comprehensive PK/PD analyses.