In-vitro modelling of the bactericidal activity of teicoplanin versus flucloxacillin as used in surgical prophylaxis, against *Staphylococcus aureus*

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The bactericidal activities of teicoplanin and flucloxacillin in a 50:50 mixture of human serum and Iso-sensitite broth were compared in an in-vitro pharmacokinetic model, at serum concentrations present during surgical prophylaxis. The bactericidal activity of teicoplanin with and without serum was also compared. Six strains of *Staphylococcus aureus* were tested. The bactericidal rate of teicoplanin in serum was significantly lower than the rate in broth alone. However, there was no significant difference in the bactericidal rates in serum of teicoplanin compared with flucloxacillin, an antibiotic which is commonly used as prophylaxis for certain surgical procedures.

**Introduction**

Teicoplanin is a glycopeptide antibiotic active against most Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA). In animal studies, antibiotics administered more than 3 h after bacterial contamination have no observable effect on the development of wound infection. The lowest rate of surgical wound infection in clean and clean-contaminated surgery, occurs in patients given antibiotics between 0 and 2 h before the first incision, with a significant increase in infection rate with each successive hour that antibiotics are delayed. A ntibiotics that are rapidly bactericidal may be an advantage in the setting of surgical prophylaxis.

Teicoplanin kills bacteria slowly and is highly protein-bound. In time-kill studies the bactericidal rate of teicoplanin against staphylococci and streptococci is significantly lower in the presence of serum than in broth alone. The slow bactericidal rate in the presence of serum has been put forward as a possible reason for the decreased efficacy of teicoplanin relative to other antibiotics in experimental endocarditis, and cardiac surgery prophylaxis.

Flucloxacillin is also highly protein-bound, and is successfully used in the prophylaxis of certain surgical procedures. The in-vitro bactericidal rate of teicoplanin and flucloxacillin were adjusted hourly to simulate the pharmacokinetics previously found in patients having antibiotic prophylaxis for burns and cardiac surgery. The bactericidal rate of teicoplanin in the absence of serum is also presented.

**Materials and methods**

**Bacterial strains**

Six strains of *S. aureus* were obtained from blood cultures taken during surgery to burns in-patients not receiving antibiotic prophylaxis. One strain was methicillin-resistant. MICs were determined in Iso-sensitite broth (Oxoid, Basingstoke, UK) by the macrodilution (tube) method.

**Bactericidal curve determination**

One colony of each organism was emulsified in 20 mL of Iso-sensitite broth, and incubated overnight to give a suspension of $10^9$ cfu/mL. This suspension was diluted 1:10 in Iso-sensitite broth and then approximately 25 μL was added to 20 mL of Iso-sensitite broth alone, or 50:50 human serum (Sigma Chemical Company, Poole, UK): Iso-sensitite broth, to give approximately $10^9$ cfu/mL. This was confirmed by removing an aliquot and serially diluting the suspension (neat, 1:10 and 1:100) to give a countable number of colonies in 50 μL plated out on Iso-
sensitest agar using a spiral plater (Don Whitley Scientific Ltd, Shipley, UK). The broths (Iso-sensitest alone and 50:50 human serum:Iso-sensitest) containing 10^5 cfu/mL were then incubated at 37°C in a shaking water bath for 2 h to give a starting culture in exponential growth. This was again confirmed by removing an aliquot and plating out appropriate dilutions (neat, 1:10 and 1:100) using the spiral plater. A additional broth was placed in the water bath to warm. Stock solutions of teicoplanin (Hoechst Marion Roussel Ltd, Denham, Uxbridge, UK) and flucloxacillin (Smith-Kline Beecham Ltd, Welwyn Garden City, UK) were made up from powder of known potency, with sterile distilled water, then further diluted in Iso-sensitest broth or 50:50 human serum:Iso-sensitest broth as appropriate. Stock solutions and human serum were stored at 70°C until needed.

For each test organism, 3.5 mL of the serum:broth mixture was placed in a tube with 0.5 mL of teicoplanin (400 mg/L) and 0.5 mL of flucloxacillin (250 mg/L) for the kill curve determination, and 4 mL of the serum:broth was placed in a sterile tube to act as control. The tubes were mixed by swirling and returned to the water bath. After 1, 2, 3, 4 and 5 h, 1 mL volumes of the kill broths and control broths were removed for plate counts. This was replaced with the appropriate amount of warmed serum:broth mixture to obtain the relevant concentration of teicoplanin or flucloxacillin required at that time in the kill tube. The same quantity of sterile warm serum:broth was added to the control tube. The tubes were returned to the water bath between sampling.

The 1 mL aliquots removed from the kill tubes were spun at 13,200 g for 5 min in Eppendorf tubes, the supernatant was discarded to remove the antibiotic, and the same amount of sterile serum:broth was replaced. The tubes were then vortexed to resuspend the organisms and the process was repeated. The control tubes were treated identically. The final resuspended broth was diluted and plated out using the spiral plater. The procedure was repeated for teicoplanin in Iso-sensitest broth without serum.

The concentrations of teicoplanin chosen were the mean concentrations previously measured in 20 burn patients following a 12 mg/kg single iv dose at these times,\(^9\) and the flucloxacillin concentrations used were obtained from pharmacokinetic data obtained during cardiac surgery.\(^7\)

The plates were incubated overnight at 37°C and counts were performed on the plates. The counts from three plates at dilutions for each aliquot, were counted. The plates were then reincubated for a total of 48 h and rechecked for additional growth that may have occurred from antibiotic-damaged organisms. Adjustments were made to the counts if additional growth had occurred. The limit of the assay was 1 cfu per 50 µL or \(\log_{10} 1.3\).

### Statistical methods
Non-parametric distributions were described by a median and range. Differences between medians of actual bacterial counts were tested by the Mann–Whitney U-test\(^{10}\) (Minitab Statistical Software 8.21).

### Results
Five methicillin-sensitive \(S. aureus\) (MSSA, strains 301, 302, 359, 422 and 509) and one MRSA (strain 847) were obtained from the blood of burn patients during surgery to the burn wound. The concentrations of teicoplanin and flucloxacillin used versus time are shown in Table I. The minimum inhibitory concentrations determined for the six organisms are shown in Table II. The bactericidal activity of teicoplanin against the six strains of \(S. aureus\) as determined in Iso-sensitest broth without added serum, is shown in Figure 1. The bactericidal activity of teicoplanin versus flucloxacillin against the five strains of MSSA and one strain of MRSA as determined in a 50:50 mixture of Iso-sensitest broth with human serum, were performed in parallel, and are shown in Figures 2 and 3.

#### Table I. Teicoplanin and flucloxacillin concentration profiles

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Teicoplanin</th>
<th>Flucloxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>concentration (mg/L)</td>
<td>volume of 50:50 serum:broth added (mL)</td>
</tr>
<tr>
<td>0–1</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>1–2</td>
<td>35</td>
<td>1.3</td>
</tr>
<tr>
<td>2–3</td>
<td>26</td>
<td>1.1</td>
</tr>
<tr>
<td>3–4</td>
<td>21</td>
<td>0.8</td>
</tr>
<tr>
<td>4–5</td>
<td>19</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\(^{a}\) Add 0.5 mL of teicoplanin 400 mg/L.

\(^{b}\) Add 0.5 mL of flucloxacillin 240 mg/L.
Bactericidal rate of teicoplanin versus flucloxacillin

There was no significant difference in the number of cfu present at the time of addition of antibiotic between the three sets of curves. Significantly fewer bacteria were killed by teicoplanin in the presence of serum than by teicoplanin in broth alone at 1 h (P = 0.005), 2 h (P = 0.005), 3 h (P = 0.005), 4 h (P = 0.016) and 5 h (P = 0.008) (Mann–Whitney). However, there was no significant difference in the log kill between teicoplanin and flucloxacillin in serum, at 1, 2, 3, 4 or 5 h. Taking 3 h as including the length of most operations, the median kill of teicoplanin in broth alone was 3.19 log_{10} i.e. 99.9% kill. The median kill at 3 h for teicoplanin in serum was 1.95 log_{10} (range 1.12–2.56) while that for flucloxacillin in serum was 2.10 log_{10} (range 1.97–2.51) (no significant difference) (Table III). For teicoplanin in 50% serum, the lowest log_{10} kill at 1, 2, 3 and 4 h was against the MRSA strain.

Figure 1. Viable counts in log_{10} cfu/mL against time in hours, after exposure of six strains of S. aureus in Iso-sensitest broth alone (●), and to reducing concentration of teicoplanin (■) similar to those in surgical prophylaxis. The solid line joins the median viable count of the six strains at each sampling time and the vertical lines show the range of viable counts.

Figure 2. Viable counts in log_{10} cfu/mL against time in hours, after exposure of five strains of methicillin-sensitive S. aureus in a 50:50 mixture of Iso-sensitest broth and human serum, to a pharmacokinetic model of teicoplanin and flucloxacillin as used in surgical prophylaxis. ●, No antibiotic; ■, with teicoplanin; ▲, with flucloxacillin.

Figure 3. Viable counts in log_{10} cfu/mL against time in hours, after exposure of a methicillin-resistant strain of S. aureus in a 50:50 mixture of Iso-sensitest broth and human serum, to a pharmacokinetic model of teicoplanin and flucloxacillin as used in surgical prophylaxis. ●, No antibiotic; ■, with teicoplanin; ▲, with flucloxacillin.

Table II. MICs (mg/L) of teicoplanin and flucloxacillin for the six organisms under study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Teicoplanin</th>
<th>Flucloxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>301</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>302</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>359</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>422</td>
<td>1.0</td>
<td>0.25</td>
</tr>
<tr>
<td>509</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>847</td>
<td>0.5</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

Table III. Log_{10} kill (median and range) of S. aureus by teicoplanin and flucloxacillin. Differences between teicoplanin in 50% serum and flucloxacillin in 50% serum were not significant (Mann–Whitney) (flucloxacillin versus MRSA excluded)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Teicoplanin</th>
<th>Flucloxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4 (1.5–3.7)</td>
<td>0.5 (0.3–1.2)^a</td>
</tr>
<tr>
<td>2</td>
<td>2.8 (2.3–3.3)</td>
<td>1.2 (0.6–1.7)^a</td>
</tr>
<tr>
<td>3</td>
<td>3.0 (2.5–3.7)</td>
<td>2.0 (1.1–2.6)^a</td>
</tr>
<tr>
<td>4</td>
<td>3.7 (2.2–5.3)</td>
<td>2.3 (2.0–4.0)^a</td>
</tr>
<tr>
<td>5</td>
<td>4.5 (2.1–6.0)</td>
<td>2.8 (1.8–3.7)</td>
</tr>
</tbody>
</table>

^a The lowest figure in the range is for the MRSA strain.
Discussion

In the presence of falling concentrations similar to those observed during surgery, both teicoplanin and flucloxacillin achieved a median 100-fold bactericidal kill in 50% serum in 3 h against M SSA. As has been noted previously, teicoplanin killed M RSA more slowly and further study is warranted. Growth of the M RSA strain was apparently inhibited in the presence of flucloxacillin and serum. Most M RSA are heterogeneous in the expression of resistance, and growth at 37°C rather than 30°C could have suppressed the resistant phenotype.

The bactericidal rate of penicillins and glycopeptides is time-dependent and lower than that of the aminoglycosides or quinolones, in which concentration rather than duration is important. Earlier pharmacokinetic studies showed that, throughout surgery, both teicoplanin (12 mg/kg iv) and flucloxacillin (500 mg iv) have serum concentrations that exceed by ten-fold the MIC for the strains of S. aureus in this investigation. The only exception was the methicillin-resistant strain with respect to flucloxacillin. Successful prophylaxis requires these serum concentrations to be sufficient to kill the contaminating bacteria during, rather than after, surgery. Antibiotics given later have little effect in preventing wound infection.

Teicoplanin and flucloxacillin are both highly protein-bound (93% and 94%, respectively) and, since it has been suggested that only the unbound fraction can interact with bacteria, protein binding might reduce penetration of antibiotic to the site of infection. The rate of killing of S. aureus by teicoplanin has been found to be reduced in the presence of serum and to be lower than that by vancomycin which is only 34% protein bound in 50% bovine serum. The reduced free concentration of teicoplanin can be overcome by raising the dose of teicoplanin but only at concentrations higher than those achieved clinically. Previous studies have used arbitrary constant antibiotic concentrations and have not compared teicoplanin with an antibiotic with similar protein binding.

Although it is well established that there is an effect of protein binding on antibacterial activity in vitro, the effect in vivo is unclear. Whether bactericidal rate influences success following antibiotic prophylaxis is yet to be explored, but it is probably only one of a number of factors involved, including penetration to the site required, area under the concentration-time curve, and the MICs for the contaminating organisms. When used for the prevention of wound infection in cardiac surgery, teicoplanin (6 mg/kg) was less successful than a combination of flucloxacillin and tobramycin, most failures being due to susceptible coagulase negative staphylococci. Low tissue concentrations may have been to blame and teicoplanin has been successful in orthopaedic and vascular surgery in which staphylococci are the important pathogens. In burns surgery, teicoplanin (12 mg/kg) did not affect the postoperative clinical course but did reduce the number of wounds from which staphylococci were isolated (unpublished observations).

A single dose of 6 mg/kg of teicoplanin appears sufficient to prevent wound infection in vascular and orthopaedic surgery even though fat concentrations were as low as 1.6 mg/L. In cardiac and possibly burns surgery, penetration to the site of bacterial seeding may be insufficient, possibly because the contaminating inoculum is greater and contains more sub-populations with reduced susceptibility to teicoplanin. A dose of 12 mg/kg in these patients would at least ensure that the concentration of teicoplanin in fat exceeds the likely MIC for staphylococci. Alternatively, as has been demonstrated in the treatment of staphylococcal endocarditis, combination with one or two doses of gentamicin would allow the maximum bacterial killing in high risk indications. Penetration of flucloxacillin into fat is similarly poor following a single dose but, if it is combined with an aminoglycoside, the rate of wound infection is kept low.

The study confirmed that the bactericidal rate of teicoplanin is significantly slower in the presence of serum. However, at operative concentrations in vitro, it does not appear that teicoplanin is at any significant disadvantage in serum against MSSA compared with flucloxacillin. Teicoplanin is also bactericidal against M RSA, but probably more slowly.

References

Bactericidal rate of teicoplanin versus flucloxacillin


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