Effect of human plasma on the antimicrobial activity of iclaprim *in vitro*

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**Objectives:** Iclaprim is a novel diaminopyrimidine for which a human plasma binding level of ~93% has been reported. The purpose of this study was to evaluate the effect of human plasma on the *in vitro* activity of iclaprim and to compare it with that of fusidic acid, teicoplanin and vancomycin, antibiotics with protein binding to human plasma of 97%, >90% and 55%, respectively.

**Methods:** MICs were determined using 40 methicillin-susceptible *Staphylococcus aureus* (MSSA) and 38 methicillin-resistant *S. aureus* (MRSA) isolates in Mueller–Hinton broth (MHB) alone or in the presence of 50% human plasma.

**Results:** MICs of iclaprim were not affected by the addition of human plasma. MIC ranges (MIC$_{90}$) for iclaprim against MSSA and MRSA were 0.016–0.06 mg/L (MIC$_{90}$ 0.06 mg/L) and 0.016–0.5 mg/L (MIC$_{90}$ 0.06 mg/L), respectively, in MHB and 0.016–0.125 mg/L (MIC$_{90}$ 0.06 mg/L) and 0.016–0.25 mg/L (MIC$_{90}$ 0.125 mg/L), respectively, in the presence of human plasma. As expected, the antimicrobial activity of fusidic acid was greatly affected by the presence of human plasma (MIC elevations of 4- to >128-fold), whereas MICs of vancomycin remained unchanged. By contrast, despite the high protein binding, MICs of teicoplanin were only marginally affected by the presence of plasma with an MIC elevation of maximum 8-fold for two strains.

**Conclusions:** This study demonstrates that human plasma does not affect the MIC of iclaprim *in vitro*.

Keywords: antibiotics, MICs, MRSA, protein binding, *Staphylococcus aureus*

**Introduction**

The novel 2,4-diaminopyrimidine iclaprim specifically and selectively inhibits bacterial dihydrofolate reductase, a key enzyme in the bacterial folate pathway. Iclaprim is an extended-spectrum antibiotic that targets severe infections requiring hospital treatment, such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA) including strains resistant to other antibiotic classes. Iclaprim also exhibits potent activity against the respiratory tract pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila* and *Chlamydia pneumoniae*. Intravenous iclaprim has recently completed two pivotal Phase III trials for the treatment of complicated skin and skin structure infections (www.arpida.com).

Plasma protein binding is often, though not always, associated with a certain loss in microbiological activity. For example, MICs of fusidic acid (97% plasma protein bound) have been reported to be only marginally affected by human plasma and changes in MIC are comparable to those of vancomycin, which has low plasma protein binding (~55%). The aim of this study was to determine the effect of human plasma on the MIC of iclaprim (~93% plasma protein bound) using a panel of *S. aureus* strains in comparison with fusidic acid, teicoplanin and vancomycin.

**Materials and methods**

**Bacterial strains**

Organisms used were from the Arpida strain collection and included clinical isolates from different countries in Europe and North America and type strains. Seventy-eight strains of *S. aureus* [40 methicillin-susceptible *S. aureus* (MSSA) and 38 MRSA] were tested including the quality control strains *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213.

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Antimicrobial agents

Vancomycin and oxacillin were purchased from Fluka (Sigma-Aldrich, Buchs, Switzerland); fusidic acid (FUS) was purchased from Sigma (Sigma-Aldrich); and teicoplanin was from Apin Chemicals Ltd (Abingdon, UK). Iclaprim (ICL) was from Arpida AG (Reinach, Switzerland).

MIC determination

MICs were determined following the standard CLSI protocol using doubling dilutions of iclaprim or fusidic acid (0.016–16 mg/L). Other reference antibiotics were tested from 0.125 to 128 mg/L. CLSI breakpoints were used to classify resistance to oxacillin. Microbroth dilutions were performed in cation-adjusted Mueller–Hinton broth (MHB; Oxoid, Basingstoke, UK) in the absence and presence of 50% whole human plasma (PAA Laboratories GmbH, Pasching, Austria). After incubation for 16–20 h at 37°C in ambient air, the MIC was determined as the lowest concentration of drug that led to no visible growth. The effect of human plasma on the activity of oxacillin was only investigated for the 40 MSSA strains.

Results

MIC data are summarized in Table 1. The activity of iclaprim was similar against MSSA and MRSA with MICs ranging from ≤0.016 to 0.5 mg/L in the absence of human plasma. In the presence of 50% human plasma, MICs of iclaprim were similar to those observed in the absence of plasma. The highest MIC of iclaprim in the presence of plasma was 0.25 mg/L (Table 1).

MICs of fusidic acid were affected the most by the presence of human plasma in the test medium and were 4- to ≥128-fold greater against 75/77 strains (excluding one fusidic acid-resistant MSSA).

The antimicrobial activity of teicoplanin was marginally affected by human plasma and the geometric mean MICs of teicoplanin were ~3-fold greater in the presence of plasma for MSSA and MRSA (Table 1). The majority of strains exhibited teicoplanin MICs that were one (37/78) or two (30/78) dilutions greater in the presence of plasma. In contrast, MICs of vancomycin were not generally antagonized by human plasma and geometric mean MICs were similar in the absence and presence of plasma (Table 1).

Table 1. MIC50s, MIC90s, MIC ranges and geometric mean MICs of iclaprim and reference antibiotics for 40 MSSA and 38 MRSA isolates (MIC in MHB/MIC in MHB with 50% human plasma); MICs are in mg/L.

<table>
<thead>
<tr>
<th>Organism/antibiotic</th>
<th>MIC50</th>
<th>MIC90</th>
<th>MIC range</th>
<th>Geometric mean MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSSA (40)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICL</td>
<td>0.03/0.03</td>
<td>0.06/0.06</td>
<td>≤0.016–0.06/≤0.016–0.125</td>
<td>0.04/0.04</td>
</tr>
<tr>
<td>VAN</td>
<td>1/1</td>
<td>2/2</td>
<td>1–2/0.5–2</td>
<td>1.15/1.30</td>
</tr>
<tr>
<td>TEC</td>
<td>0.5/2</td>
<td>1/2</td>
<td>≤0.125–2/≤0.125–8</td>
<td>0.74/1.95</td>
</tr>
<tr>
<td>FUS</td>
<td>0.06/2</td>
<td>0.25/16</td>
<td>≤0.016–16/0.25 to &gt;16</td>
<td>0.50/5.38</td>
</tr>
<tr>
<td>OXA</td>
<td>0.5/1</td>
<td>1/2</td>
<td>0.25–1/0.25–4</td>
<td>0.62/1.04</td>
</tr>
<tr>
<td><strong>MRSA (38)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICL</td>
<td>0.03/0.06</td>
<td>0.06/0.125</td>
<td>≤0.016–0.5/≤0.016–0.25</td>
<td>0.05/0.06</td>
</tr>
<tr>
<td>VAN</td>
<td>1/2</td>
<td>2/4</td>
<td>0.5–8/1–8</td>
<td>1.54/1.97</td>
</tr>
<tr>
<td>TEC</td>
<td>0.5/2</td>
<td>2/4</td>
<td>0.25–8/0.5–32</td>
<td>1.19/3.26</td>
</tr>
<tr>
<td>FUS</td>
<td>0.125/4</td>
<td>0.25/4</td>
<td>≤0.016–0.25/0.5–16</td>
<td>0.13/3.88</td>
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<tr>
<td>OXA</td>
<td>128/—</td>
<td>&gt;128/—</td>
<td>4 to &gt;128/—</td>
<td>94.21/—</td>
</tr>
</tbody>
</table>

ICL, iclaprim; VAN, vancomycin; TEC, teicoplanin; FUS, fusidic acid; OXA, oxacillin.

Discussion

Iclaprim exhibited potent activity against 40 MSSA and 38 MRSA strains with MICs ranging from ≤0.016 to 0.06 mg/L for MSSA (MIC50 0.06 mg/L) and <0.016 to 0.5 mg/L for MRSA (MIC50 0.06 mg/L) in the absence of plasma. Notably, iclaprim MICs in the presence of plasma were the same for all strains tested with the exception of four strains. Three strains exhibited a two dilutions’ greater MIC of iclaprim in the presence of plasma, whereas one strain demonstrated a two dilutions’ lower MIC with plasma. These data show that human plasma did not significantly affect the MIC of the drug.

As expected from its low protein binding (~55%), MICs of vancomycin were also unaffected by the presence of human plasma. As for iclaprim, the MICs of vancomycin in the presence of plasma were very similar for all strains tested except for three, all of which exhibited a two dilutions’ greater MIC in the presence of plasma. Although protein binding of >90% has been reported for teicoplanin,6 MICs were only marginally affected by the presence of human plasma in the growth medium, which is in agreement with reported data. For instance, Chambers and Kennedy10 reported a 4-fold greater MIC for a clinical isolate of S. aureus in the presence of 50% rabbit serum.

In contrast, the activity of fusidic acid was up to 128-fold lower in the presence of plasma. Fusidic acid is known to be 97% protein bound6 and exhibits elevated MICs in the presence of serum. For instance, Somekh et al.6 found an up to 700-fold increase in the MIC of fusidic acid by adding 50% human serum for MSSA and MRSA; another study reported an increase in MIC50 for MRSA from 4 to 256 mg/L by the addition of 50% heat inactivated human serum.7
In conclusion, three different antibiotics with similar protein binding [iclaprim (~93%), teicoplanin (>90%) and fusidic acid (97%)] were compared with an antibiotic with much lower protein binding [vancomycin (~55%)]. The effects of human plasma on the antimicrobial activity of these antibiotics show marked differences in that iclaprim was similar to vancomycin and teicoplanin, antibiotics whose MICs are either not affected or minimally affected, whereas the activity of fusidic acid was markedly affected. These data suggest that despite the observed protein binding of iclaprim its in vitro antimicrobial activity is maintained in the presence of human plasma, which is suggestive of a weak and loose association of the drug with plasma proteins.

Further studies investigating the affinities and capacities of each antibiotic to plasma proteins and the effects of plasma on the bactericidal properties and other pharmacodynamic parameters (such as a post-antibiotic effect) would be helpful in fully understanding why plasma affects some, though not all, of the antibiotics in this study.

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Transparency declarations

All authors are employees of Arpida AG, Reinach, Switzerland, and own/have owned stocks or shares in Arpida AG.

References