Results and Discussion

Background: The antibacterial activity of DAP is diminished by interaction with pulmonary surfactant, which is the reason why DAP cannot be used in community-acquired pneumonia. The in vitro inhibition of antibacterial activity of FRI by pulmonary surfactant was much less pronounced than DAP which decreased 256-fold (Figure 3).

Methods

Bacterial strains: A total of 33 Gram-positive aerobes, comprising clinical isolates and quality control strains were used for the development and validation of MIC methods: 6 E. faecalis (including 2 VanB); 3 E. faecium (1 VanA, 1 VanB); 13 S. aureus (4 MSSA, 9 MRSA, 6 GISA); 6 coagulase-negative Staphylococci (1 NSBE), 2 S. epidermidis (1 FQ1), 1 S. haemolyticus (NG25).

Susceptibility tests: MICs were determined by standard broth microdilution (BMD) in cation-adjusted Mueller-Hinton broth (CAMHB) according to CLSI guidelines. For standard testing of FRI and DAP the calcium content of MH broth was adjusted to a final concentration of 50 mg Ca²⁺ yielding CAMHB50. For the FRI reference method CAMHB50 was supplemented with 0.002% (v/v) polysorbate 80 (Tween80). The influence of pulmonary surfactant on the antibacterial activity of FRI and comparator drugs was determined by BMD, supplementing CAMHB50 with Survanta® (Abbott GmbH & Co. KG, Wiesbaden, Germany) to final concentrations of 0.25%, 0.5%, 1%, 5%, 10%, and 15% (v/v). Due to the turbidity of the medium at concentrations >1% (v/v) Survanta®, MIC values were read as color change from yellow to purple-blue, after the addition of fuchsins/iodine/iodine potassium titrate (MFT, final concentration 0.5 mg/ml) to the test wells and a further incubation at 35°C for 45 min. FRI was obtained from Combinature Biopharm AG, Berlin, Germany, and other drugs from their respective manufacturers.

Results and Discussion

Influence of Ca²⁺ concentrations on MIC

The antibacterial activity of FRI and DAP against S. aureus ATCC29213 and E. faecium ATCC19434 was strongly dependent on the presence of free Ca²⁺ ions. A Ca²⁺ concentration of 50 mg/L (corresponding to physiological concentration in blood), FRI displayed strong activity which reached its maximum at 100 mg Ca²⁺/L. For DAP, the highest activity against E. faecium was reached at 200 mg Ca²⁺/L.

Table 1: MICs (mg/L) at different Ca²⁺ concentrations

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Ca²⁺ concentration (mg/L)</th>
<th>MIC (mg/L)</th>
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<tbody>
<tr>
<td>Daptomycin</td>
<td>0</td>
<td>&gt;25</td>
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<td></td>
<td>20</td>
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<td></td>
<td>50</td>
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<td></td>
<td>100</td>
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<tr>
<td>VANCOMYCIN</td>
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<td>&gt;25</td>
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Optimal procedure for MIC determination of FRI

MIC determination of FRI is complex, since results are strongly influenced by the method (agar dilution showed a tendency to lower values against aerobic Gram-positive compared to BMD) and by the final concentration of Ca²⁺ and Tween80. The optimal procedure (reference method) for determining the MIC of FRI is BMD with MH broth supplemented with Ca²⁺ of 50 mg/L and 0.002% (v/v) Tween80. Under these conditions FRI MIC values against 33 selected aerobic Gram-positive bacteria are comparable with DAP (Table 2).

Conclusions

• BMD using MH broth containing 50 mg/L Ca²⁺ and 0.002% (v/v) Tween80 is the optimal procedure to determine MICs for FRI
• Use of FRI reference method gives MICs against 33 aerobic Gram-positive bacteria comparable to DAP
• In contrast to DAP, FRI is not significantly inhibited by high inoculum densities or the presence of high pulmonary surfactant concentrations

Literature


Fig. 1 Friulimicin B

Fig. 2: In vitro effect using S. aureus ATCC29213