Caspofungin (CAS), Colistin (CST), and Polymyxin B (PMB) Enhance Moxifloxacin (MXF) Activity against Staphylococcus aureus Biofilms by Disturbing Matrix exopolysaccharides
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ABSTRACT

Objective: Biofilms are poorly responsive to anti-staphylococcal antibiotics. Our aim was to examine whether combining MXF with amphiphilic drugs without anti-staphylococcal activity such as CST, PMB or CAS, could improve MXF activity against S. aureus biofilms.

Methods: Biofilms were grown for 24 in 96-well plates in Tryptic Soy Broth (TSB) supplemented with 1% NaCl and 1% glucose. 24h-old (maximal) biofilms were exposed for 48 h to a wide range of MXF conc. (to obtain full conc.-response curves), combined with CAS, CST, or PMB at 2x MXF. Viability in biofilms was quantified using the redox indicator resazurin [1]. MXF penetration in biofilms was assessed by confocal microscopy [2, 3] and Albalasmeh et al. [4] EPS macromolecular size was measured by dynamic light scattering.

Results: CAS, CST, and PMB had MICs of 4, 50, and 50 mg/L, respectively and did not show any activity by themselves against biofilms at 2x MXF. MXF alone was poorly active (50% reduction in viability reached only for ATCC29213 and 1 clinical isolate at conc. 20mg/L). Combination with CAS, CST or PMB showed reaching 50 % reduction in viability for 6/7, 3/7 and 4/7 clinical strains respectively, CAS or CST increased up to 30-fold MXF penetration, reduced diameter, 40% EPS content, and decreased at least 60% EPS size for biofilms formed by strain 2005/179 but not from 2003/651.

Conclusion: Amphiphilic molecules like CAS, PMB or CST reduce EPS content and size in biofilms, which may contribute to enhance MXF penetration and activity. Combination with amphiphiles appears thus as a promising strategy to act upon S. aureus biofilms, warranting the search of more potent molecules.

INTRODUCTION

• The ability of S. aureus to produce biofilms is considered as a main reason for persistence or recurrence of infections. Biofilm formation is a multi-step process, which involves bacterial attachment to a support, formation of complex aggregates of adhering microorganisms, and production of a matrix rich in exopolysaccharides (EPS).

• Within biofilms, bacteria are poorly responsive to antibiotics. We showed that biofilms formed by clinical isolates of S. aureus are particularly reluctant to antibiotic action [1]. Now therapeutic strategies are therefore needed to try improving antibiotic activity against these specific forms of infection.

• Antiseptic agents are in general much more active against biofilms, possibly due to their amphipathic character. Yet they cannot be used for deep tissues infections because of their intrinsic toxicity.

OBJECTIVE

• The aim of our study was to examine whether combining an anti-staphylococcal antibiotic with amphiphilic drugs showing no intrinsic activity on staphylococci, like the anti-gram-negative polymyxin B (PMB) and colistin (CST) or the antifungal caspofungin (CAS), may improve activity on preformed biofilms.

• To this end, we used moxifloxacin as an exemplar antibiotic and biofilms formed by 7 clinical isolates (epidemic clones isolated from various body sites) + a reference strain [1] + 2005/179 but not from 2003/651.

• Data were correlated with changes in moxifloxacin penetration within biofilms and in biofilm exopolysaccharides (EPS) content and size.

RESULTS

MICs in broth and relative potency of moxifloxacin alone or in combination with CAS, PMB, CST against mature biofilms

<table>
<thead>
<tr>
<th>strain</th>
<th>MXF MIC (mg/L)</th>
<th>MXF</th>
<th>MOF</th>
<th>MOF+CAS</th>
<th>MOF+PMB</th>
<th>MOF+CST</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC29213 (MRSA)</td>
<td>0.032</td>
<td>4</td>
<td>0.1</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Surv2003/651 (MSSA)</td>
<td>1.75</td>
<td>16</td>
<td>8</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Surv2001/1083 (MSSA)</td>
<td>0.125</td>
<td>1</td>
<td>0.68</td>
<td>0.9</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Surv2001/209 (MSSA)</td>
<td>1.75</td>
<td>&gt;20</td>
<td>2</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

Concentration-response curves: viability (% control values) in biofilms exposed to moxifloxacin alone or in combination for 2 exemplative clinical isolates

Influence of CAS, CST, PMB on EPS content (A) and EPS size (B) in biofilms of strains 2005/179 or 2003/651

METHODS

• Biofilms were cultured in polystyrene 96-well plates in TSB; 2% NaCl; 1% glucose at 37°C for 24h with an initial inoculum adjusted to an OD620nm of 0.05. Biofilms were exposed to increasing concentrations (0-25;30mg/L) of moxifloxacin alone or with combination with CAS (80 mg/L), CST (100mg/L), PMB (100mg/L) during 48h. Biofilm mass was evaluated by measuring the OD of crystal violet and viability of bacteria, using the redox indicator resazurin (reduced to fluorescent resorufin by viable bacteria) [1].

• EPS content was assessed by confocal microscopy and Albalasmeh et al. [4] EPS macromolecular size was measured by dynamic light scattering.

• EPS macromolecular size was measured by dynamic light scattering.

• EPS content and size in biofilms were exposed to increasing concentrations (0-25;30mg/L) of moxifloxacin alone or with combination with CAS (80 mg/L), CST (100mg/L), PMB (100mg/L). Combination with CAS, CST or PMB allowed reaching 50 % reduction in viability for 6/7, 3/7 and 4/7 clinical strains respectively. CAS, CST or PMB increase up to 30-fold MXF penetration, reduced diameter, 40% EPS content, and decreased at least 60% EPS size for biofilms formed by strain 2005/179 but not from 2003/651 strain.

• CAS, CST, PMB reduce exopolysaccharides (EPS) content and size in 2005/179 biofilm but not in 2003/651 strain.

CONCLUSION

• Caspofungin, colistin and polymyxin B, can reduce EPS content and size in S. aureus biofilms, which enhances moxifloxacin diffusibility and activity in these biofilms.

• The reasons why some clinical isolates remain poorly susceptible to these effects need to be further investigated.

REFERENCES