A laboratory study of the synergistic effect of chlorhexidine and silver

Kristina Blom¹ and Maria Werthén²

¹Medibiome, Mölndal, Sweden, ²Mölnlycke Health Care, Gothenburg, Sweden

ABSTRACT

Healthcare-associated infections (HAIs) are a significant economic burden on healthcare systems. Skin antisepsis is a key factor in preventing many of these HAIs associated with skin microorganisms. However, the persistence of bacteria on the skin after antisepic treatment and the bacteria’s ability to repopulate these treated areas over time¹ suggest that, in cases where the skin is compromised, infection remains a risk.

Combination therapy may be used to extend the range over which antimicrobials work and may help to prevent the emergence of resistant strains and provide a synergy between antimicrobials.²⁻¹

The combination of chlorhexidine diacetate and silver sulphate was evaluated in a checkerboard assay. These substances were found to have synergistic activity against four different isolates of Pseudomonas aeruginosa and one strain of Methicillin-resistant Staphylococcus aureus.

METHODS

MICROORGANISMS: Four strains of Pseudomonas aeruginosa: ATCC 15692 (PAO1), ATCC 15442, isolate A, and isolate B and one strain of Methicillin-resistant Staphylococcus aureus (MRSA). Bacterial suspensions were prepared by inoculating colonies to Mueller-Hinton Broth (MHB) followed by overnight incubation at 37°C. Susensions were adjusted to a working dilution of 1-2x10⁶ CFU/ml.

CHECKERBOARD TEST: Minimum Inhibitory Concentration (MIC) analyses were done to determine the MICs of chlorhexidine diacetate (CHA) and silver sulphate (Ag₂SO₄) alone and in combination in a checkerboard assay. In the checkerboard assay, 96 well plates are used where one chemical is diluted from top row to bottom row and the other chemical from first to last column. Bacterial suspension at the same concentration is then added. Controls for no growth and growth are included. Inhibition of growth was evaluated by measuring optical density at 595nm after the plate had been incubated at 35°C for 24 hours. To assess the synergistic activity of antimicrobial combinations, the Fractional Inhibitory Concentration Index (FICI) was determined using the following formula:

\[
FICI = \frac{MIC_{CHA} \text{ for CHA alone}}{MIC_{CHA} \text{ for CHA in combination}} + \frac{MIC_{Ag2SO4} \text{ for Ag2SO4 alone}}{MIC_{Ag2SO4} \text{ for Ag2SO4 in combination}}
\]

The combination was considered synergistic when the FICI was ≤0.5, indifferent when the FICI was >0.5 to <4, and antagonistic when the FICI was >4.

RESULTS

The values of the MICs of CHA and Ag₂SO₄ against the 5 bacterial strains are presented in Table 1 and 2. The checkerboard assay was repeated at lower dilutions to find the lowest dilution that gives inhibitory effect when the antimicrobial agents are combined (Table 2). The data show that the combination of CHA and Ag₂SO₄ demonstrated synergistic activity against all strains of bacteria. That is, there was a reduction observed in the CHA and Ag₂SO₄ concentrations required to inhibit P. aeruginosa and MRSA when these antimicrobial agents were combined, compared with their activity when assayed in isolation (Table 1 and 2, Figure 1).

Table 1. MIC and checkerboard assay assessment of interaction between CHA and Ag₂SO₄.

<table>
<thead>
<tr>
<th>Specie</th>
<th>Strain</th>
<th>MICCHA (µg/ml)</th>
<th>MICAg₂SO₄ (µg/ml)</th>
<th>FICI</th>
<th>Synergistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>ATCC 15692 (PAO1)</td>
<td>22</td>
<td>≤1.4</td>
<td>10</td>
<td>≤2</td>
</tr>
<tr>
<td></td>
<td>ATCC 15442</td>
<td>44</td>
<td>≤1.4</td>
<td>10</td>
<td>≤2</td>
</tr>
<tr>
<td></td>
<td>Clinical isolate 10.5</td>
<td>44</td>
<td>≤1.4</td>
<td>10</td>
<td>≤2</td>
</tr>
<tr>
<td></td>
<td>Clinical isolate 13.1</td>
<td>44</td>
<td>1.4</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>MRSA</td>
<td>CCG 35600</td>
<td>0.7</td>
<td>≤1.4</td>
<td>2.5</td>
<td>≤2</td>
</tr>
</tbody>
</table>

MIC = Minimum Inhibitory Concentration, CHA = Chlorhexidine diacetate, FICI = Fractional Inhibitory Concentration Index, NPC = Not Possible to Calculate.

Table 2. Checkerboard assay assessment at lower dilutions than presented in table 1 to find the lowest dilution that gives inhibitory effect when the antimicrobial agents are combined against P. aeruginosa and MRSA.

<table>
<thead>
<tr>
<th>Specie</th>
<th>Strain</th>
<th>MICCHA (µg/ml)</th>
<th>MICAg₂SO₄ (µg/ml)</th>
<th>FICI</th>
<th>Synergistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1</td>
<td>ATCC 15692</td>
<td>22</td>
<td>≤0.14</td>
<td>10</td>
<td>0.39</td>
</tr>
<tr>
<td>MRSA</td>
<td>CCG 35600</td>
<td>0.7</td>
<td>0.05</td>
<td>2.5</td>
<td>0.63</td>
</tr>
</tbody>
</table>

MIC = Minimum Inhibitory Concentration, CHA = Chlorhexidine diacetate, FICI = Fractional Inhibitory Concentration Index.
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\section*{RESULTS}

\begin{itemize}
  \item Chlorhexidine diacetate in combination with silver sulphate inhibited growth of \textit{P. aeruginosa} at \( \leq 0.14 \) µg/ml and alone at 22 µg/ml – i.e. at more than 157 times lower concentration → FICI ≤0.5.
  \item Chlorhexidine diacetate in combination with silver sulphate inhibited growth of MRSA at 0.05 µg/ml and alone at 0.7 µg/ml – i.e. at 14 times lower concentration → FICI ≤0.5.
  \item Silver sulphate in combination with chlorhexidine diacetate inhibited growth of \textit{P. aeruginosa} at 0.39 µg/ml and alone at 10 µg/ml – i.e. at 26 times lower concentration → FICI ≤0.5.
  \item Silver sulphate in combination with chlorhexidine diacetate inhibited growth of MRSA at 0.63 µg/ml and alone at 2.5 µg/ml – i.e. at 4 times lower concentration → FICI ≤0.5.
\end{itemize}

\section*{CONCLUSIONS}

\begin{itemize}
  \item The results (FICI ≤0.5) demonstrated that combining chlorhexidine diacetate (CHA) with silver sulphate (Ag\textsubscript{2}SO\textsubscript{4}) improved the antimicrobial activity of both CHA and Ag\textsubscript{2}SO\textsubscript{4} against four different strains of \textit{P. aeruginosa} and MRSA with both antimicrobial agents showing synergism when combined.
  \item These results suggest improved skin antisepsis when these antimicrobial agents are used in combination in a cover dressing.
\end{itemize}

\begin{figure}
\centering
\includegraphics{figure1.png}
\caption{MIC values for CHA and Ag\textsubscript{2}SO\textsubscript{4} assessed alone and in combination. *Synergy with FICI ≤0.5.}
\end{figure}

\section*{REFERENCES}