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KOLUMAN, AHMET; AKTAŞ, ALPER; and ADİLOĞLU, SELEN (2022) "Antimicrobial activities of Ankaferd BloodStopper, hypochlorous acid, and chlorhexidine against specific organisms," Turkish Journal of Medical Sciences: Vol. 52: No. 1, Article 31. https://doi.org/10.3906/sag-2107-24
Available at: https://journals.tubitak.gov.tr/medical/vol52/iss1/31

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Antimicrobial activities of Ankaferd BloodStopper, hypochlorous acid, and chlorhexidine against specific organisms

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To the Editor,

Many antimicrobial agents can be used for the control of bacterial colonization, plaque accumulation, inflammation, and postoperative infection after the oral surgery [1]. It had been hypothesized to compare the effects of Ankaferd BloodStopper (ABS), hypochlorous acid (HOCl), and chlorhexidine gluconate (CHG) on specific microorganisms such as Streptococcus mutans, Staphylococcus aureus, Actinomyces israelii, and Lactobacillus casei to find ideal antimicrobial agent.

In this hypothesis, it was seen that higher concentration and long exposure time of all agents are more impressive at all of the microorganisms. In the long-term exposure, it was found that HOCl and CHG affect faster than ABS for Lactobacillus casei. For A. israelii, the effect of CHG is more than ABS and HOCl (CHG > HOCl > ABS) for all organisms at short-term exposure. However, in long-term exposure, HOCl is more effective for L. casei and S. aureus than ABS but for A. israelii and S. mutans, there was not a significant difference. Although the effect of CHG on S. mutans started in 120 s, ABS and HOCl needed more time to affect. Coleghon et al. showed that CHG had a larger inhibition zone than ABS at both long- and short-term exposure similar to our study [2]. In the long-term, effectiveness of CHG continued for all microorganisms in this study (Table 1) (Figure).

In disc diffusion, lower concentrations of all agents were more effective for microorganisms. ABS had better results for A. israelii and L. casei than other agents. At disc diffusion, CHG was worse than ABS and HOCl (Table 2).

Barry et al. applied CHG on 1100 strains of gram-positive and gram-negative bacteria and the resistance to CHG is seen low [3]. Sensitivity of Streptococcus mutans to CHG is more than sensitivity of Lactobacillus species to CHG dealing with pH level [4]. In this study, pH was not examined but a significant difference was not seen between L. casei and S. mutans according to the concentration and duration. Only the concentration of CHG is found as important for the efficiency on S. mutans group.

Cinar et al. showed that ABS had a smaller inhibition zone but at long-term exposure, the results of ABS do not differ from those of the other antimicrobial agents [2]. ABS has an additional hemostatic and antiinflammation effect, beside antimicrobial effect [5]. In this study, the time needed for the effectiveness of ABS is observed to be more than CHG and HOCl. On the other hand, ABS disc diffusion form can be preferred due to its superiority to CHG and HOCl.

ABS has pleiotropic effects on blood cells, vascular endothelium, angiogenesis, and cellular proliferation that can help the tissue regeneration and wound healing [6]. In the literature, it was shown that ABS deactivates the proliferation of the cell lines and cancer cells [7]. Therefore, the combined effect (antimicrobial, hemostatic, and improving wound accelerator) makes ABS more valuable than its equivalents.

A possible advantageous use of ABS for human health was seen on Helicobacter pylori, an antibiotic resistant species shown in the literature [8]. If ABS is so effective on a resistant microorganism like H. pylori at high concentration without any damage to the living tissues, this antibacterial agent can be a good choice for precaution of the other resistant microorganisms.

Cinar et al. observed that Lactobacillus species have a resistance to ABS [2]. In our study, L. casei was resistant to ABS in short term exposure, but it was seen that in long-term exposure and at high concentration, the effect of the ABS did not differ from that of CHG and HOCl. ABS had the same effect with CHG and HOCl for A. israelii in long-term exposure with high concentration but there is not sufficient research in the literature about the effect of ABS on Actinomyces species.

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Received: 04.07.2021 • Accepted/Published Online: 17.10.2021 • Final Version: 22.02.2022

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Table 1. The effect of ABS, CHX, and HOCl on different bacterial species in short- and long-term.

<table>
<thead>
<tr>
<th></th>
<th>Control population</th>
<th>Ankaferd (%)</th>
<th>CHG (%)</th>
<th>HOCl (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>100</td>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>Streptococcus mutans ATCC 25175</td>
<td>5th min</td>
<td>7.819544</td>
<td>5.255273</td>
<td>6.60206</td>
</tr>
<tr>
<td></td>
<td>10th min</td>
<td>3.748188</td>
<td>4.78533</td>
<td>2.963788</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 23235</td>
<td>5th min</td>
<td>7.880814</td>
<td>5.892095</td>
<td>6.986772</td>
</tr>
<tr>
<td></td>
<td>10th min</td>
<td>3.857332</td>
<td>4.826075</td>
<td>2.939519</td>
</tr>
<tr>
<td>Actinomyces israelii (Kruse) Lachner-Sandoval ATCC 10049</td>
<td>5th min</td>
<td>7.431364</td>
<td>5.70757</td>
<td>6.832509</td>
</tr>
<tr>
<td></td>
<td>10th min</td>
<td>3.39794</td>
<td>4.70757</td>
<td>2.792392</td>
</tr>
<tr>
<td>Lactobacillus casei ATCC 334</td>
<td>5th min</td>
<td>7.653213</td>
<td>5.838849</td>
<td>6.431364</td>
</tr>
<tr>
<td></td>
<td>10th minute</td>
<td>3.929419</td>
<td>5.94939</td>
<td>2.944483</td>
</tr>
</tbody>
</table>

Green = Active biocidal applications decrease at least 3 log according to CLSI and EUCAST
Yellow = Insufficient applications defined by CLSI and EUCAS.

![Graph](image-url)

Figure. The graphic of the effects of antimicrobial agents on bacterial species.

Table 2. The effect of disc diffusion of ABS, CHX, and HOCl on bacterial species.

<table>
<thead>
<tr>
<th></th>
<th>Ankaferd (%)</th>
<th>CHX (%)</th>
<th>HOCl (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>Streptococcus mutans ATCC 25175</td>
<td>18</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 23235</td>
<td>19</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Actinomyces israelii (Kruse) Lachner-Sandoval ATCC 10049</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Lactobacillus casei ATCC 334</td>
<td>9</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>

Yellow = Resistant or intermittent
Green = Sensitive
HOCl is an important antimicrobial agent that is synthesized with acidification of H₂O₂. In this research, it was seen that HOCl is effective on S. aureus more than ABS, less than CHG. Also, when the concentration (200 ppm) is higher, the efficiency of HOCl is better. Ishihara et al. showed that when applying 100 ppm HOCl, chlorine levels can reduce by the presence of organic compounds. Therefore, if concentration is higher, chlorine level and effectiveness can be higher [9].

As a result, in long-term exposure, 3 of the agents have similar antimicrobial effects and can be preferred successfully during oral surgery. Clinician should keep in mind the tissue-friendly character of HOCl, wound healing effect of ABS, and antiplaque activity of CHX while choosing the agent to prefer for the oral surgery patients.

**Conflict of interest**
The author declares no financial or other conflicts of interest related to this paper.

**References**