ANTIBACTERIAL EFFECTS OF VARIOUS CHEMICAL AGENTS ON AGGREGATIBACTER ACTINOMYCETEMCOMITANS

Abstract

Aim: Periodontal diseases are chronic, inflammatory, and infectious diseases. Therefore, periodontal treatment aims to eliminate periodontopathic bacteria causing periodontal diseases. The aim of the present study was to evaluate the effect of commonly-used products such as acetic acid, sodium bicarbonate, and sodium chloride on periodontopathic bacteria, Aggregatibacter actinomycetemcomitans. Material and Method: In the present research, effects on Aggregatibacter actinomycetemcomitans (ATCC 33384TM) were tested. Acetic acid, sodium bicarbonate, and sodium chloride were used in 5 concentration dissolved in distilled water. The antibacterial efficacy against bacteria was tested via disc-diffusion method, minimum inhibitory concentration test, and minimum bactericidal concentration tests. Results: The most antibacterial efficacy was found in ciprofloxacin. Penicillin had moderate effect and chlorhexidine provided a similar efficacy. Acetic acid provided an inhibitory effect higher than penicillin and chlorhexidine against Aggregatibacter actinomycetemcomitans but lower than tetracycline and ciprofloxacin. Sodium bicarbonate and sodium chloride showed no inhibitory effect. Discussion: Acetic acid is commonly consumed as mouthwash, dentifrice, gel, and/or irrigation agent is necessary. Keywords: Aggregatibacter Actinomycetemcomitans, Aggressive Periodontitis, Acetic Acid; Oral Care.

Keywords

Aggregatibacter Actinomycetemcomitans, Aggressive Periodontitis, Acetic Acid; Oral Care.
**Introduction**

Periodontitis is the chronic inflammatory and infectious disease of periodontium primarily caused by dental plaque [1]. There are two forms of the disease: chronic periodontitis (CP) and aggressive periodontitis (AgP) [2]. In both forms, bacterial accumulation initiates an inflammatory process and host-bacterial interactions determine the disease course [3]. In AgP, both the bacterial component of dental plaque and the response to these bacteria is different from CP, resulting in a more rapid and severe disease course [2]. AgP lesions are usually associated with a certain bacterial strain called Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) [4, 5]. Because of these differences between the diseases, the treatment modalities are also different. CP generally responds well to conventional periodontal treatment while AgP requires additional applications such as chemotherapeutic agents [6]. These chemotherapeutics include mainly antibiotics and antiseptics such as chlorhexidine. However, long-term use of these agents causes adverse effects like antibiotic resistance, suppression of regular oral microbiota, and fungus superinfection. Therefore, naturally-derived agents that do not cause these adverse effects might be beneficial.

Antibiotics act by disrupting genetic materials, cell wall, or metabolism [7]. Chlorhexidine, on the other hand, acts by binding cationic chlorhexidine molecules to the cell wall [8]. Most of the bacteria are sensitive to the environmental changes. Thus, changes in temperature, pH, pressure, and/or humidity can also inhibit bacterial growth. For instance, acidic compounds increase pH and most bacteria cannot grow in an acidic medium. Acetic acid is an organic compound with a pH 2.4 and is used in many households as vinegar. Sodium bicarbonate is a food additive that increases pH due to its alkalinity. Acetic acid has been shown to have antibacterial efficacy by various studies [9] while sodium bicarbonate has been shown to exhibit antifungal properties [10, 11]. As for sodium chloride, bacterial growth requires certain amounts of sodium chloride and concentration in the growth medium influences bacterial growth. High concentrations of sodium chloride in an environment might provide antibacterial efficacy by causing lysis [12]. Acetic acid, sodium bicarbonate, and sodium chloride are commonly-used products that, due to their chemical structures, might exhibit antibacterial properties against periodontopathogenic bacteria. Therefore, the aim of the present study was to evaluate the effect of these products on A. actinomycetemcomitans cell culture via the Kirby-Bauer test, also called the disc-diffusion test.

**Material and Method**

Acetic acid (Sigma, St. Louis, Missouri, USA), sodium bicarbonate (Sigma, St. Louis, Missouri, USA), and sodium chloride (Sigma, St. Louis, Missouri, USA) were used as test materials. Penicillin, ciprofloxacin, tetracycline, and chlorhexidine (CHX) were used as positive controls and distilled water was used as a negative control. All solutions except CHX were prepared as 5% dilutions of each material in distilled water. 0.012% CHX was used. The antibacterial efficacy of test materials was tested via the Kirby-Bauer (disc-diffusion) method and minimum inhibitory concentration and minimum bactericidal concentrations were also determined.

**Disc-diffusion method [13]**

The bacterial species used in this study was A. actinomycetemcomitans (ATCC 33384TM). The antimicrobial activity was determined with the disc-diffusion method. First, nutrient agar (NA) was prepared and 108 CFU/mL of bacteria was added to 100 mL NA solution. Then, bacteria was inoculated to the petri dish containing Mueller-Hinton agar (MHA) medium, which does not include any indicator or inhibitor. 38.0 g/L MHA was sterilized by autoclave (121°C, 15 min). After cooling to 45-50 °C 5% defibrinated sheep blood was added. 20 mL of blood-enriched MHA was poured into sterile petri dishes. The blank discs (6 mm diameter, Oxoid) were impregnated with 20 mL of each test compound dissolved in distilled water (105 µg/disc) and placed on the inoculated agar. The inoculated plates were incubated at aerobic conditions with 36°C for 24 h. After incubation, the growth inhibition zones were measured via a millimetric scale. The procedure was repeated two more times and the arithmetic mean of the three measurements was recorded as one inhibition zone. The results are shown in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Materials</th>
<th>A. actinomycetemcomitans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition zones</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 mm</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>17 mm</td>
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<tr>
<td>Metronidazole</td>
<td>X</td>
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<tr>
<td>Ciprofloxacin</td>
<td>48 mm</td>
</tr>
<tr>
<td>Chlorhexidine 0.12%</td>
<td>11 mm</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>X</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>12 mm</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>X</td>
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</tbody>
</table>

**MIC tests**

MIC values of test materials against A. actinomycetemcomitans were determined with a micro-well dilution method (Figure 1). Tryptic soy broth (TSB) was used in MIC tests. TSB: 20 gr tryptone (Sigma, St. Louis, Missouri, USA); 5 gr soytone (Sigma, St. Louis, Missouri, USA); 5 gr NaCl and 950 ml distilled water were mixed to form a 30 gr/L solution that was then sterilized with autoclave (121°C, 15 min). After cooling to 47°C, 5.0 µg/ml hemin and 0.5 µg/ml vitamin K1 were added and gently mixed. The inoculum of microorganisms was prepared using 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. Test compounds and the positive control agents dissolved in distilled water were first diluted to the high-
Porphyromonas gingivalis, and Fusobacterium nucleatum, pro-
derived from AgP lesions, to A. actinomycetemcomitans. Results demonstrated that the major ingredient of vinegar, acetic acid, has a strong antibacterial efficacy while neither sodium bicarbonate nor salt had an inhibiting effect. Treatment of aggressive periodontitis is one of the most challenging practices for clinicians. There are no established protocols and guidelines for effective and complete treatment of the disease [14]. The most accepted treatment measures combine conventional mechanical, nonsurgical, and surgical treatments with diverse adjunctive anti-infective therapies such as anti-septics and antibiotics [15]. As A. actinomycetemcomitans can invade gingival tissues and is generally related to AgP lesions, systemic and/or local antibiotics are usually recommended as adjuncts to conventional treatment. However, there are certain disadvantages of chemotherapy. These are bacterial resistance, adverse systemic effects of systemic antibiotics, cost of local antibacterial agents, and alteration in regular oral microbial components [16]. The present study evaluated the effective-ness of acetic acid, bicarbonate, and salt as antiseptic and antibacterial agents that are natural products with low side effects.

Most of the bacterial species in the oral cavity are anaerobic and/or facultative anaerobic bacteria [17]. These bacteria obtain their energy from phosphorylation at the substrate level and produce metabolic end products such as long, medium, and short chain fatty acids [18]. These by-products inhibit metabolism and growth of other bacterial cells and even the host defense mechanism [19, 20]. Acetic acid is a short chain fatty acid (SCFAs) produced by bacteria as an end metabolite. Periodontopathogenic bacteria, A. actinomycetemcomitans, Porphyromonas gingivalis, and Fusobacterium nucleatum, pro-
duce SCFAs as metabolic products [21]. Recently acetic acid has been reported to inhibit the growth and biofilm formation of the strongly pathogenic bacterium Pseudomonas aeruginosa [22]. Changes in local tissue concentrations of SCFAs are related to the metabolism of the dysbiotic microbiota and infectious diseases such as periodontitis alter the concentrations of these molecules. In the present study, 5% acetic acid exhibited more antibacterial efficacy than CHX and penicillin against A. actinomycetemcomitans. In contrast, Huang et al. reported that acetic acid had no significant antibacterial effect on A. actino-
mycetemcomitans [23]. Other than acetic acid, sodium bicarbonate is also known as an antimicrobial agent especially effective on fungus. Research has shown that bicarbonate inhibits growth of C. albicans [24]. Nonetheless, sodium bicarbonate was found to be ineffective as an antibacterial agent [10]. Likewise, our present results found no inhibitory effect of sodium bicarbonate on A. actinomy-
cetemcomitans. Another household agent tested in this study was sodium chloride. Bacteria usually do not require sodium ions for growth, and high concentrations of salt inhibit bacterial growth (except halophilic or halotolerant species). However, antibacterial tests showed that no inhibition zone was observed with a 5% concentration of salt.

Conclusions
Among the tested molecules, only acetic acid showed antibac-
terial effectiveness against A. actinomycetemcomitans. Acetic acid is a commonly-used product and has no side effects with low doses such as a 5% concentration. Due to its biological properties, use of acetic acid as an irrigation agent and/or mouthwash might be beneficial as an adjunctive agent to peri-
odontal therapy.

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Competing interests
The authors declare that they have no competing interests.

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