ANTIBACTERIAL EFFECTS OF VARIOUS CHEMICAL AGENTS ON AGGREGATIBACTER ACTINOMYCETEMCOMITANS

FARKLI KİMYASAL AJANLARIN AGGREGATİBACTER ACTİNOMYCETEMCOMİTANS BAKTERİSİ ÜZERİNDEKİ ANTİBAKTERİYEL ETKİLERİNİN ARAŞTIRILMASI

ANTIBACTERIAL EFFECTS OF CHEMICAL AGENTS ON AGGREGATIBACTER ACTINOMYCETEMCOMITANS

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Öz

Amaç: Periodontal hastalıklar kronik, enflamatuvar ve enfeksiyöz karakterde hastalıklardır. Bu sebeple periodontal tedavi hastalığa neden olan periodontopatojen bakterilerin eliminasyonunu hedefler. Bu araştırmanın amacı, doğal kimyasal ürünler olan asetik asit, sodyum bikarbonat ve sodyum klorürün Aggregatibacter actinomycetemcomitans üzerindeki etkilerinin araştırılmasıdır. Gereç ve Yöntem: Bu araştırmada Aggregatibacter actinomycetemcomitans (ATCC 33384TM) suşu test edilmiştir. Asetik asit, sodyum bikarbonat ve sodyum klorür, %5 konsantrasyonda distile suda dilüe edilerek uygulanmıştır. Negatif kontrol olarak distile su, pozitif kontroller olarak penisilin, tetrasiklin, siprofloksasin ve %0,12'lik klorheksidin, kullanılmıştır. Test ajanlarının antimikrobiyal etkileri, disk difüzyon, minimal inhibitör konsantrasyon ve minimal bakterisidal konsantrasyon testleri ile araştırılmıştır. Bulgular: Antibakteriyel etkinliği en fazla olan antibiyotik siprofloksasin bulunmuştur. Penisilin, A. actinomycetemcomitans üzerine orta seviyede etki göstermiştir. Klorheksidin penisiline benzer bir etki göstermiştir. Asetik asit, klorheksidin ve penisilinden daha kuvvetli bir inhibitor etki sağlamıştır. Sodyum bikarbonat ve tuz antibakteryel etki sağlamamıştır. Tartışma: Asetik asit anti-bakteriyel bir metabolittir ve aynı zamanda sirke olarak günlük tüketimi vardır. Periodontal patojen bakteriler üzerindeki antibakteriyel etkisi sayesinde, periodontal tedaviye yardımcı olabilir. Gargara, diş macunu, jel veya irrigasyon ajanı şeklinde preparatları hazırlanarak klinik kullanıma uygunluğunun test edilmesi gereklidir.

Anahtar Kelimeler

Aggregatibacter Actinomycetemcomitans; Agresif Periodontitis; Ağız Sağlığı; Asetik Asit

Abstract

Aim: Periodontal diseases are chronic, inflammatory, and infectious diseases. Therefore, periodontal treatment aims to eliminate periodontopathogenic 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 periodontopathogenic 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 negative control agent was distilled water and the positive control agents were chlorhexidine, penicillin, tetracycline, and ciprofloxacin. 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 in the form of vinegar. Due to its antibacterial efficacy against Aggregatibacter actinomycetemcomitans, it can be useful as an adjunct to periodontal treatment. Further studies to evaluate clinical use of acetic acid as mouthwash, dentifrice, gel, and/or irrigation agent are necessary.

Keywords

Aggregatibacter Actinomycetemcomitans; Aggressive Periodontitis; Acetic Acid; Oral Care;

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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.

Materials	A. actinomycetemcomitans		
	Inhibition zones	MIC values	MBC values
Penicillin	10 mm	MIC was not detected in 50-0.0243 dilutions.	X
Tetracycline	17 mm	MIC was not detected in 50-0.0243 dilutions.	X
Metronidazole	X	X	X
Ciprofloxacin	48 mm	3.905 µl/ml	3.905 µl/ml
Chlorhexidine 0.12%	11 mm	MIC was not detected in 50-0.0243 dilutions.	X
Sodium bicarbonate	X	X	X
Acetic acid	12 mm	7.81 µl/ml	1000 µl/ml
Sodium chloride	X	X	X

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

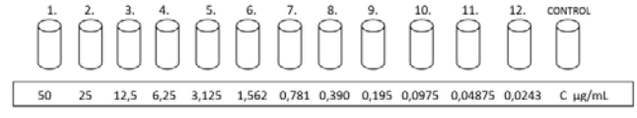

Figure 1. Minimum inhibitory concentration test protocol.

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-

est concentration tested (1000 mg/ml), and then serial two-fold dilutions were made (concentration range 7.8–1000 mg/ml) in sterile 10-ml test tubes containing TSB. 96-Well plates were prepared by dispensing 95 ml of TSB and 5 ml of the inoculum into each well. Then, 100 ml of compound solutions were added. Wells containing 195 ml of TSB without compound and 5 ml of the inoculum were used as negative control. The final volume in each well was 200 ml. The 96-well plates were incubated at 368 for 24 h. The assay was performed in triplicate.

MBC tests

Samples were taken from MIC test tubes and inoculated on petri dishes containing MHA. The lowest concentration inhibiting bacterial growth was recorded as MBC.

Results

Against A. Actinomycetemcomitans the most effective antibiotic was ciprofloxacin and second was tetracycline. The antibacterial effects of penicillin, CHX, and acetic acid were similar. There was no inhibitory effect observed in sodium bicarbonate and sodium chloride. Chlorhexidine, penicillin, and tetracycline did not exhibit antibacterial effect on MIC test tubes with the studied concentrations from 0. The results are shown in Table 1.

Discussion

The present study evaluated any possible antibacterial effects of the commonly-used products vinegar, sodium bicarbonate, and salt against one of the etiological factors of aggressive periodontitis, 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 antiseptics 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 chemotherapeutic therapy. 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 effectiveness 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, produce 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. actinomycetemcomitans [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. actinomycetemcomitans. 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 antibacterial 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 periodontal therapy.

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Competing interests

The authors declare that they have no competing interests.

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For books: Savaş Z, Batuhan M, editors. Vascular and endovascular surgery. London: W. B. Saunders; 1999. p. 87-93
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