ORIGINAL ARTICLES

The Antimicrobial Effect Of Commercial Acetic Acid Against Enterococcus Faecalis

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ABSTRACT

Aim: To study and evaluate the antimicrobial effect of commercial acetic acid (vinegar) in different concentrations on Enterococcus faecalis (E. faecalis) after five minutes and 24 hours of application, comparing with tetracycline-citric acid, 2.5% sodium hypochlorite and saline solutions.

Materials used are: commercial vinegar containing 5% acetic acid at 5, 4, 2.5 and 1% concentrations; a mixture of tetracycline-citric acid solution; 2.5% sodium hypochlorite, and normal saline solutions. 1ml of each of the tested materials was added to 5 µl of the Enterococcus faecalis inoculum in 1 ml of brain heart infusion broth, incubated overnight at 37°C, in an atmosphere of 5% CO2. Subcultures were done on blood agar plates pre and post incubation; the number of colonies after five minutes and after 24 hours were recorded.

Results: The relative reduction percent of the number of colonies after five minutes application of the tested materials was calculated and statistically analysed using one way ANOVA and Bonferroni tests. Sodium hypochlorite gave the highest significant reduction level compared with all other tested materials (P<0.0001). Commercial acetic acid at 2.5, 4 and 5% gave very high significant reduction values (P<0.0001) after five minutes. No significant differences between 4 and 5% acetic acid (P>0.05). The relative reduction per cent after acetic acid application at all concentrations used showed high significance when compared with the tetracycline-citric acid solution (P<0.0001). After 24 hours, uncountable number of colonies was observed on specimens treated with normal saline, while no growth was found with all other tested materials.

Conclusion: Vinegar can be used as an antimicrobial agent against Enterococcus faecalis, however, more investigations are needed to prove its effectiveness as a root canal irrigant.

Key words: Acetic acid, Enterococcus faecalis, antimicrobial effect.

Introduction

The requirements for an ideal root canal irrigant are its ability to properly disinfect the root canal system, as well as to remove the smear layer, to be biocompatible to the dental supporting tissues, with a non-estranging taste or odor.

Sodium hypochlorite solution (NaOCl) is the widely used irrigating solution due to its inhibitory effect against a wide range of microorganisms (Ercan et al., 2004); however, it is unable to remove the smear layer, thus this solution alone is unable to disinfect the dental tubules unless a chelating agent is used before tubular disinfection. NaOCl becomes toxic to the vital tissues if accidentally pushed beyond the root canal space and it has an unpleasant odor and taste. Instrumentation and irrigation with NaOCl alone is not effective to disinfect the root canal system (Bystrom and Sundqvist, 1981) since the canals are left contaminated with inter tubular E. faecalis. Elimination of the microorganism is greatly improved when it is combined with ethylene diamino tetra acetate (EDTA) (Bystrom and Sundqvist, 1985).

Enterococcus faecalis, has been shown to be a pathogen that persists in the dental tubules of the medicated root canals, and has been considered to be the most common cause for periradicular lesions in post-treatment endodontic failures (Rocas et al., 2004).

Several agents have been used to disinfect the intertubular dentin; one of them was calcium hydroxide, which was ineffective, or of low action against E. faecalis (Mickei et al., 2003). Ozonated water, has a strong oxidizing power, and can act against E. faecalis by destroying the cell wall and cytoplasmic membrane (Nagayoshi et al., 2004); however it cannot reach the tubular microbes unless the smear layer has been removed.

Tetracycline when combined to citric acid and a detergent (MTAD), introduced by Torabinejad et al Torabinejad et al., 2003), was effective in removing the smear layer, and an effective antimicrobial compound against the intertubular E. faecalis with the use of NaOCl final rinse (Shabahang et al., 2003; Shabahang and Torabinejad 2003).

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Commercial acetic acid (vinegar), a 100% volatile acidic solution, has been applied into several fields as an antimicrobial agent; it is widely used in food industry to inhibit yeast metabolism (Silva et al., 2004), used in medical field to treat otitis externa (Balen et al., 2003; Benson, 1998), and in the management of wounds heavily colonized with Pseudomonas aeruginosa and Candida albicans (Bowler et al., 2001); however it has not been reported to be used as an intracanal medicament.

The aim of the present study was to evaluate the antimicrobial effect of a commercial acetic acid (vinegar) in different concentrations on E. faecalis, in comparison to tetracycline-citric acid solution (TCS), and 2.5% sodium hypochlorite solutions.

Materials and Methods

Bacteriologic preparation:

Freshly isolated known strain of E. faecalis, was obtained from the Microbiology Laboratory, Maternity and Children’s Hospital, Dammam, KSA. Identification of the strain was done by gram stain, which showed short gram positive cocci in pairs and short chains, catalase negative, bile esculin positive and by Vitek2 (automated identification and susceptibility system, bioMe’rieux)

Inoculum preparation:

A broth suspension of E. faecalis standardized to 0.3 McFarland turbidity standard (optical density at 570 nm), with concentration of 10^8 CFU/ml. (Murray, 2007, CLSI, 2009), was prepared as the inoculum used in the study.

A total of forty two, 2 ml graduated sterile test tubes, were used in this study. These were divided into seven groups of 6 samples each. The first four groups were used to test the effect of the different concentrations of the acetic acid (1, 2.5, 4 and 5%); the fifth group for the tetracycline-citric acid solution (TCS), the sixth for the sodium hypochlorite 2.5% which served as positive control; and a seventh negative control group for normal saline solution.

Under aseptic condition, each test tube was filled with 1 ml of brain heart infusion broth, and 5 µl of the inoculum, incubated overnight at 37°C, in an atmosphere of 5% CO2.

Materials preparation:

Commercial vinegar (American Garden, NY, USA) containing 5% acetic acid (AA) buffered at pH 2.75 was used in the study at 5, 4, 2.5 and 1% concentrations.

A fresh tetracycline-citric acid solution (TCS) was prepared by adding three equal volumes of tetracycline solution at pH 2.0 to citric acid solution (Julfar, Ras Al Khaimah, UAE) at pH 6.0 and normal saline solution. The tetracycline solution was prepared by dissolving tetracycline powder (Julfar, Ras Al Khaimah, UAE) in 10 ml of distilled water with constant stirring until saturation.

Testing procedure:

A subculture from each tube was taken directly on a sheep blood agar plate, to ensure bacterial growth, and to count the number of growing colonies before the application of the tested materials. 1ml of each tested materials was added to one group of the inoculums. Subcultures were done after 5 minutes and 24 hours on sheep blood agar plates by using sterile loops (µl); all plates were incubated overnight at 37 °C in CO2 incubator. These two time points were selected to resemble the clinical condition of the irrigation time in case of one visit and in multi-session endodontic treatments.

To calculate the amount of changes after application of each tested material, colonies of bacteria were counted from the subcultures of all groups.

The effects of the different materials, on the studied bacteria, were evaluated by calculating the relative reduction percent in the number of colonies by using the equation:

$$R = \frac{(A - B) \times 100}{A}$$

- **R** = Relative reduction percent
- **A** = The mean count of colonies before application
- **B** = The mean count of colonies after application
The results were recorded, tabulated and statistically analysed. Statistical analysis was carried out using Instant2 program on the collected data. ANOVA (analysis of variance) was used to test the difference in the mean number of colonies among more than two groups. Multiple comparisons were performed using Bonferroni test to detect the significant difference between the groups. The level $P \leq 0.05$ was considered the value of significance.

**Results:**

The mean and standard deviation of the number of colonies, before application of the tested materials, after five minute and after 24 hour application periods, are presented in Table 1.

The differences in the relative reduction in colonies number between the different groups were tested using one-way analysis of variance. Multiple comparisons were performed using Bonferroni test at 95% confidence level. (Table 2)

There was extremely significant variation between the relative reduction in colonies number between the seven groups, $F=1078 & P<0.0001$.

Group AA 1% reported statistical significance of an average 6.6 less than group AA 2.5% ($P < 0.05$). Statistically very highly significant an average 16.7, 21.7 & 66.9 less than group AA4%, AA 5% & NaOCl 2.5% respectively, and statistically very high significance of an average 12.3 & 12.3 higher than group TCS & Saline respectively ($P<0.0001$).

Group AA 2.5% reported statistically very high significance of an average 10.1, 15.1 & 60.3 less than group AA4%, AA 5% & NaOCl 2.5% respectively, and statistically very high significance of an average 18.9 & 87.1 higher than group TCS & Saline respectively ($P<0.0001$).

Group AA 4% reported statistically very high significance of an average 5.00 less than group AA 5% ($P>0.05$), statistically very high significance of an average 50.2 less than NaOCl 2.5% group ($P<0.0001$), and statistically very high significance of an average 34.00 & 102.2 higher than group TCS & Saline respectively ($P<0.0001$).

Group AA 5% reported statistically very high significance of an average 54.2 less than NaOCl 2.5% group ($P<0.0001$), and statistically very high significance of an average 29.00 & 97.2 higher than group TCS & Saline respectively ($P<0.0001$).

**Table 1:** The relative reduction percent (R) and standard deviation (SD) in all groups, after five minutes and 24 hours of application.

<table>
<thead>
<tr>
<th>Material</th>
<th>Before application</th>
<th>After 5mn</th>
<th>After 24hr</th>
<th>Relative reduction % (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA 1%</td>
<td>235</td>
<td>157.2</td>
<td>0</td>
<td>34.8 (+, 4.7) 100</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>350</td>
<td>211.4</td>
<td>0</td>
<td>39.5 (+, 8.5) 100</td>
</tr>
<tr>
<td>AA 4%</td>
<td>220</td>
<td>110.3</td>
<td>0</td>
<td>49.8 (+, 1.4) 100</td>
</tr>
<tr>
<td>AA 5%</td>
<td>419</td>
<td>189.4</td>
<td>0</td>
<td>53.8 (+, 7.2) 100</td>
</tr>
<tr>
<td>TCS</td>
<td>225.6</td>
<td>178.6</td>
<td>0</td>
<td>20.8 (+, 6.2) 100</td>
</tr>
<tr>
<td>NaOCl 2.5%</td>
<td>218</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Normal Saline</td>
<td>112.5</td>
<td>165.8</td>
<td>Uncountable</td>
<td>-47.4 (+, 2.4) uncountable</td>
</tr>
</tbody>
</table>

**Table 2:** The differences in the relative reduction in colonies number between the different tested groups

<table>
<thead>
<tr>
<th>(I)</th>
<th>(J)</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA 1%</td>
<td>AA 2.5%</td>
<td>-6.6(*)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AA 1%</td>
<td>AA 4%</td>
<td>-16.7(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 1%</td>
<td>AA 5%</td>
<td>-21.7(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 1%</td>
<td>TCS</td>
<td>12.3(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 1%</td>
<td>NaOCl 2.5%</td>
<td>-66.9(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 1%</td>
<td>Saline</td>
<td>80.5(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>AA 2.5%</td>
<td>-10.1(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>AA 4%</td>
<td>-15.1(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>AA 5%</td>
<td>-20.8(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>TCS</td>
<td>18.9(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>NaOCl 2.5%</td>
<td>-60.3(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 2.5%</td>
<td>Saline</td>
<td>87.1(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 4%</td>
<td>AA 5%</td>
<td>-5(NS)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AA 4%</td>
<td>TCS</td>
<td>29(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 4%</td>
<td>NaOCl 2.5%</td>
<td>-50.2(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 4%</td>
<td>Saline</td>
<td>97.2(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 5%</td>
<td>TCS</td>
<td>34(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 5%</td>
<td>NaOCl 2.5%</td>
<td>-45.2(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA 5%</td>
<td>Saline</td>
<td>102.2(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>TCS</td>
<td>NaOCl 2.5%</td>
<td>-79.2(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>TCS</td>
<td>Saline</td>
<td>68.2(*** )</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>NaOCl 2.5%</td>
<td>Saline</td>
<td>147.4(*** )</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
*** Very highly significant.
I= Tested materials   J= Tested groups
Group TCS reported statistically very high significance of an average 79.2 less than NaOCl 2.5% group (P<0.0001), and statistically very high significance of an average 68.2 higher than Saline group (P<0.0001).

Group NaOCl 2.5% reported statistically very high significance of an average 147.4 higher than Saline group (P<0.0001).

Discussion:

E. faecalis is a Gram-positive, facultative anaerobe microorganism, present in the endodontic microbiota, remains viable in most of the dentinal tubules of the treated root canals, and is mainly responsible for the post endodontic failures. The removal of the smear layer by a chelating agent permits the intra-canal disinfectant to penetrate the tubules, and to be in direct contact and reducing the tubular microorganisms (D’Arcangelo C et al., 1999).

The micro-organism is acid sensitive, it can survive in alkaline pH media (McHugh et al., 2004), this explains its resistance to calcium hydroxide root canal dressing (5), and its neutralization in 10% citric acid with a stabilizer (Barroso et al., 2004 and Mickei et al., 2003).

Acetic acid is widely used as an antimicrobial agent in different fields; for killing food-borne pathogenic bacteria (Berry and Cutter, 2000; Rhee et al., 2003), to inhibit Escherichia coli growth (Roe et al., 2002); used in combination with boric acid or corticosteroids to treat ear infections (Balen et al., 2003), and to disinfect contaminated wounds (Bowler et al., 2001).

Commercial acetic acid (vinegar) has not been reported to be used as an intracanal disinfecting medicament. The material is completely volatile, has a specific gravity 1.05 close to that of water, and of low pH, these properties enable it to remove the smear layer, permitting an easy flow and penetration of the material into the dental tubules without the need of a final washing solution.

Acetic acid with low pH 2.75 is used in the study to permit better chelation of calcium ions (Haznedaroglu, 2003), however, it was able to completely destroy E. faecalis after 24 hours at all concentrations used. Comparing the results between 4 and 5% acetic acid concentrations, the difference was insignificant; however it was statistically high significant when compared with the other concentrations used after five minutes. The reduction values of the different concentrations of the AA solution, is directly proportional to the concentration of the material after five minutes.

At all concentrations used, acetic acid gave statistically high significant results than the tetracycline-citric acid compound; this can be explained by the difference in pH of the two solutions.

Resembling the clinical conditions, the five minutes of bacterial contact with the chemical agent, permit disinfection of the root canal in a reasonable clinical period.

The results obtained from this study, proved that the commercial acetic acid when used alone as an intracanal irrigant, even at 1% concentration, can significantly reduce the growth of the E. faecalis after five minutes, and neutralization after 24 hours.

Further microbiologic and biologic studies are needed to evaluate the material as a multifunctional single root canal irrigating solution and its tolerability by the periapical tissue.

References


