Antibacterial Activity of Halothane, Eucalyptol and Orange Oil

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Summary

Staphylococcus aureus *and* Enterococcus faecalis *species were used for the experiment. 0,5 McFarland bacterial inoculum was diluted using saline in proportions 1:10, 1:10^2, 1:10^3, 1:10^4, 1:10^5 and 1:10^6. 0.2 millilitre of solving agent was mixed with an equal quantity of bacterial suspension. Sixty specimens, 10 for each proportion, were prepared. Specimens were hermetically closed and left for 10 and 30 minutes. 0.1 millilitre of each specimen was planted to the blood agar and put in the thermostat at 37°C for 24 hours. The procedure was repeated twice. Halothane exhibited greatest antibacterial activity, destroying all concentrations of* Staphylococcus aureus *and* Enterococcus faecalis. *Eucalyptol showed activity towards* Staphylococcus aureus*, while orange oil did not show any antibacterial effect on the examined bacteria.*

Key words: *re-treatment, halothane, eucalyptol, orange oil,* Staphylococcus aureus*,* Enterococcus faecalis*.*

Acta Stomat Croat
2000; 307-309

ORIGINAL SCIENTIFIC PAPER
Received: October 22, 2000

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Introduction

Endodontic re-treatment is indicated when the root canal filling does not satisfy the measurements of endodontic treatment. If the filling is not homogenous and post-endodontic crown care is not satisfactory; microorganisms may advance from both coronal and apical directions. Consequences of bacterial proliferation are persistent pain after the treatment, developing or persistent periapical lesions with the possibility of acute exacerbation or total destruction of the periodontal ligament (1). Bacteria may remain in the apical portion of the root canal if the filling does not reach the apical foramen. The number of isolated bacterial species is greater in treated than in untreated teeth (2-7). Microbiologic flora in treated teeth is predominantly gram-positive with equal portions of facultative and obligatory anaerobes, while endodontically untreated teeth have polymicrobial flora with equal portions of gram-positive and gram-negative bacteria. Such a finding may be consequent to the ability of some

microorganisms' to survive in a new environment, or they become resistant to disinfectants used during endodontic treatment. This ability can be found in a few species, and the most frequently noted is *Enterococcus faecalis* (8). *In vitro* studies showed this species to be resistant to calcium hydroxide (9,10,11). It has been demonstrated that enterococci can survive even without the presence of other microorganisms. *Actinomyces israelii* and *Propionobacterium propionicum* may interfere with the healing of periapical lesions if they are present in the root canal after the treatment (12,13,14). Therefore, the drugs used in re-treatment procedures should have antibacterial characteristics. Agents that are in use today are eucalyptol and halothane, although others, especially etherical oils, are being investigated.

The purpose of this study was to investigate the antibacterial characteristics of eucalyptol, halothane and orange oil *in vitro* on *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) species.

Materials and methods

Standard species of bacteria *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212, and blood agar were used for the study. Chloroform was used as the positive control, and 0.9% saline was used as the negative control.

In preparing the initial inoculum we used 24-hour standard bacteria species culture on blood agar in such a manner that 4 to 5 colonies were transported to 5 ml of 0.9% saline solution (NaCl). The thickness of suspension was 0.5 McFarland (108 CFU/ml). Standard inoculum was further diluted with saline to achieve the following pro-portions: 1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$ and $1:10^6$. Each of the six solutions were divided into 10 cuvettes, so that each cuvette was filled with 0.2 ml of the solution, using a micropipette. The same procedure was performed for both bacteria species. To each diluted suspension 0.2 ml of each of the investigated diluters was added, including the controls. Finally, we had 60 specimens for both bacteria species.

Specimens were divided in to two groups (30 for each bacteria) and left in glass cuvettes that were hermetically closed with an isolated gum cork, at room temperature, for 10 minutes for the first and 30 minutes for the second group. The specimens, including the controls, were then planted on blood agars and left to incubate at 37°C. Plantation process was as follows: 0.1 ml of each dilution was spread on a blood agar, imitating the letter "L". After drying, the agars were put into a thermostat, and after 24 hours the colonies were counted. The whole procedure was repeated once more.

Results

The results of this study show that halothane has the greatest antibacterial efficacy as a whole, like the control (chloroform). Halothane destroyed all concentrations of both *Enterococcus faecalis* and *Staphylococcus aureus* in both time period (10 and 30 minutes).

Eucalyptol showed antibacterial effect for *Staphylococcus aureus* in both time period, but not for *Enterococcus faecalis*, which grew colonies after both time periods, in the dilution of the standard inoculum, and also in the negative control.

Orange oil did not show any antibacterial effect. *Staphylococcus aureus* and *Enterococcus faecalis* colonies grew on all blood agars of the dilutions of the standard inoculum, as well as in the negative control. The results of antibacterial efficacy of the solvents can be seen in Tables 1 and 2.

Discussion

The results of the study clearly show that halotane has the same complete antibacterial efficacy as chloroform. Halothane destroyed all concentrations of *Enterococcus faecalis* and *Staphylococcus aureus*. These results are in allcordance with previous studies which reported that halothane has the ability to dilute proteins (15), and that its cytotoxicity is, according to Barbosa et al. (16) similar to that of chloroform. Barbosa et al. (16) used L929 mice fibroblasts to investigate the cytotoxicity of the most frequently used gutta-percha solvents: turpentine, chloroform and halothane. Turpentine had the greatest cytotoxicity, while chloroform and halothane caused equal cell damage.

Eucalyptus oil showed limited antibacterial efficacy in both time periods. It did show bactericidal effect on *Staphylococcus aureus*, but not on the *Enterococcus faecalis*, which grew colonies equally as in the negative control. Pattnaik et al. (17) studied antibacterial characteristics of five essential oils (eucalyptol, lemon oil, geraniol, linalool, and menthol) on 19 bacterial and 12 fungal species. The results showed that all solvents have stronger or weaker antibacterial action, with eucalyptol efficient against 16 bacterial and 7 fungal species.

Orange oil did not show antibacterial efficacy in any of the studied time periods. *Staphylococcus aureus* and *Enterococcus faecalis* were present and growing on all blood agar. All the solvents were active on the principle "all or nothing".

Of the many solvents used today chloroform has, the best antibacterial activity and is, therefore used as a positive control. Molander et al. (18) showed that in 21 cases in which chloroform was used, it significantly decreased bacterial growth, explaining this by its toxicity which therefore disables the microorganisms in their growth. Our parallel study showed that notable amounts of intracanal material are pushed over the apex when chloroform and halothane are used during endodontic re-treatment. Chutich et al. (19) measured residual volume of gutta-percha softened using chloroform, halothane and xylene and pushed over the apex. They obtained results by comparing the weight of the dilutant (before and after the treatment) that was collected in tubes hermetically attached to the apex. They concluded that the obtained doses were probably toxic. Their hepatotoxicity has also been described (20), and there are also reports showing morphological changes in mice spermatozoe (21), as well as genotoxic activity in rat kidneys (22).

Although ethereal oils exhibited weaker antibacterial activity towards the two examined bacterial species, they should be the first choice material in endodontic re-treatment. Special attention should be paid to eucalyptus oil which obtains satisfactory experimental and clinical results in time and quality of the re-treatment.