Clinical evaluation of the antibacterial action of different root canal medicaments

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ABSTRACT
Background: Different intracanal medicaments were evaluated in vivo for their antibacterial action.
Materials and methods: A total of one hundred and twenty anterior opened necrotic teeth with no apical radiolucencies were chosen for this study. Endodontic treatment procedures were performed to the teeth which were medicated between the appointments by different intracanal medicaments (Tricresol formalin, formocresol, 2% acetic acid, CMCP, calcium hydroxide, and normal saline as control). The elimination of the bacterial flora of these teeth was monitored during the treatment appointments by swabbing the root canals and culturing the swabs on blood agar culture media.
Results: The teeth medicated by tricresol formalin formocresol and 2% acetic acid experienced the most decrease in the bacteria count and showed the highest negative cultures. CMCP was next, then calcium hydroxide and lastly normal saline.
Conclusion: The chemo mechanical debridement of the root canals was effective in eliminating most of the bacterial flora but not to the degree of sterility.
Key words: endodontics, instrumentation, medicaments. (J Bagh Coll Dentistry 2011; 23(sp. issue):15-18)

INTRODUCTION
In endodontics, the use of intracanal medicaments serves the main purpose of eliminating the source of infection which is bacteria. The intracanal medicaments that are being used nowadays have varying degrees of antibacterial action, which are mostly acceptable, but there are other requirements for the clinical use of intracanal medicaments as their cytotoxicity, long term effectiveness, and mode of application.
There are many modes of application of the intracanal medicaments such as:
1) Vapor action, as the formaldehyde, and phenol containing medicament
2) Direct action
(a) Flooding the canal with the medicament example clindamycin (1), metronidazole (2) and chlorhexidine (3).
(b) Condensing the canal with the medicament as calcium hydroxide.
The highly toxic intracanal medicaments as the formaldehyde containing medicaments are applied in the root canal by a cotton pellet and rely on their potent antibacterial vapor. The germicidal vapor must dissolve in the tissue so that it becomes effective.

The dose of the medicament needs to be 100-1000 times higher than that when it is applied directly (4), so seepage of the medicament through the filling material or lateral and accessory canals is a threat to the surrounding structures.
There are some limitations in using vapor forming medicaments as the presence of protein containing compounds in the root canal as blood, tissue fluid, and the smear layer, hamper the diffusion of the medicament through the root canal space (5). Flooding the root canal with these toxic medicaments would make them in intimate contact with living tissue which would cause tissue necrosis. Calcium hydroxide has no vapor action; therefore the only way of using this medicament is by condensing it in the root canal to touch the root canal walls and bacteria.
Unfortunately, the smear layer delays the antibacterial action of Ca (OH)2, so it needs more time to eradicate the microbial flora than the other medicaments.
Acetic acid has proven that it has a good antibacterial action (6) and has no serious inflammatory reaction on living tissue (7). Therefore 2% acetic acid is going to be assessed clinically as an intracanal medicament.

MATERIALS AND METHODS
One hundred and twenty anterior opened necrotic teeth with no apical radiolucencies were chosen for this study. A rubber dam and a clamp were placed around each tooth, which was to be treated. Each tooth was disinfected by 70% alcohol before any procedure.

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1- First appointment

An access opening was performed to each tooth by a high speed turbine hand piece with copious water coolant. The roof of the pulp chamber was removed with no entrance to the pulp cavity so that the bacterial inhabitants would not be affected. A sterile cotton pellet was used to wipe the access opening to remove the residual cooling water.

A sterile file of size 25 was introduced to a length of 20mm to swab the root canal. The file was removed and placed in a sterile test tube containing 3ml. sterile thioglycollate broth for dilution of the sample.

An inoculum of 0.01ml volume was withdrawn and inoculated on a petri dish containing blood agar culture media. One cultured petri dish was incubated aerobically and another anaerobically at 37°C. After 24 hours, the grown bacterial colonies were counted and multiplied by the dilution factor (300), to get the number of bacteria per swab, as a baseline data.

An intracanal medicament of 0.025ml volume was placed in the canal by a mean of a standard sized cotton pellet, and then the tooth was sealed by zinc oxide eugenol cement.

The teeth were grouped to:
- Group 1: Twenty teeth treated with tricresol formalin, by the cotton pellet technique.
- Group 2: Twenty teeth treated with formocresol by the cotton pellet technique.
- Group 3: Twenty teeth treated with CMCP by the cotton pellet technique.
- Group 4: Twenty teeth treated with 50% Ca(OH)\textsubscript{2} slurry by condensing it in the root canal.
- Group 5: Twenty teeth treated with 2% acetic acid by flooding the canal.
- Group 6: Twenty teeth treated with sterile normal saline by flooding the canal (control).

2) Second appointment (after 3 days from the 1st appointment)

After isolation, disinfection, and removal of the temporary restoration, a sterile file of size 25 was placed in the root canal and the same bacteriological culturing was done to it to get the number of viable colonies after placing the medicament for 3 days.

Instrumentation was performed to the root canal by the step back technique up to size 60 (master apical file), when white dentin was withdrawn. The root canal was irrigated with 1 ml. sterile normal saline between each instrument. After the end of the instrumentation, sterile paper points were used to dry the root canal. Another sterile file of size 25 was placed in the root canal to swab it, and the same bacteriological culturing was placed in the root canal and the tooth was sealed by zinc oxide eugenol cement.

3) Third appointment (after 6 days from the 1st appointment)

The same technique of swabbing and bacteriological culturing was done after the disinfection, and removal of the temporary filling. The viable colonies indicated the viable bacteria after 3 days of instrumentation. The root canal was then restored endodontically with gutta percha, and Sealapex sealer.

RESULTS

The data are presented in figure 1 for the aerobic bacteria and figure 2 for the anaerobic bacteria.

1) First appointment (after access opening)

The cultivable bacteria were recognized as a baseline data and were expressed as 100%.

- The teeth that were flooded with 2% acetic acid decreased the root canal’s bacteria to 9% and 7.1% for the aerobic and anaerobic bacteria respectively. Flooding with normal saline decreased the viable bacteria to 43.8% and 43.6% for the aerobic and anaerobic bacteria respectively.

2) Second appointment

(a) Before instrumentation

Tricresol formalin showed the most inhibition of bacteria which was to 19% and 15.5% of the viable aerobic and anaerobic bacteria respectively. Two percent acetic acid showed a slight increase in the bacterial count than in the first appointment (12.8% and 9.8% of viable aerobes and anaerobes). The normal saline group presented a great increase in viable bacteria (72.5% for aerobes and 66.4% for the anaerobes).

(b) After instrumentation

After instrumentation and irrigation with sterile normal saline the number of bacteria declined to about 1/5-1/10 of the bacteria found before instrumentation in all the groups.

3) Third appointment

The bacterial count remained static with slight fluctuation except for group 6 (normal saline) which showed an increase in the viable aerobic and anaerobic bacteria to 20.6% and 18% respectively.

The reaction of the aerobic bacteria was similar to the anaerobic bacteria except in the first
appointment’s irrigation and second appointment – after instrumentation where the anaerobes were more sensitive to the in vivo procedure.  

Figure 1: The decline of the aerobic root canal microflora after treating with all the different medicaments.  

Figure 2: The decline of the anaerobic root canal microflora after treating with all the different medicaments.  

Table 1: The percentage of negative cultures gained from the in vivo human study  

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Percentage of negative cultures</th>
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<tbody>
<tr>
<td>Tricresol formalin</td>
<td>95</td>
</tr>
<tr>
<td>Formocresol</td>
<td>95</td>
</tr>
<tr>
<td>CMCP</td>
<td>70</td>
</tr>
<tr>
<td>Ca (OH)₂</td>
<td>60</td>
</tr>
<tr>
<td>Acetic acid 2%</td>
<td>90</td>
</tr>
<tr>
<td>Normal saline (control)</td>
<td>30</td>
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The percentage of gaining negative culture from the treated root canals before obturation shows the tricresol fomalin and formocresol to have the highest percentage with 95% negative cultures. Two percent acetic acid was next with 90% then CMCP with 70%, Ca (OH)₂ with 60% and finally normal saline (control) with 50%. This is presented in table 1.

**DISCUSSION**

After access opening and the initial irrigation by 2% acetic acid a marked decrease was encountered in the total count of the bacteria. This reduction in the bacterial count agrees with Hamoudi and Al-Najjar (6), who stated that acetic acid has an effective antibacterial action on all the bacteria when it is in direct contact.

Irrigation by normal saline eliminated slightly more than half the bacterial count due to the flushing action. The findings are comparable with the results of Bystrom and Sundqvist (8) who concluded that the flushing action of normal saline decreased the number of microorganisms in the root canal. However, they also concluded that the oxygen ingress in the root canal with the anaerobic environment was responsible for the decrease in the bacterial count.

In the second appointment, the vapor forming medicaments (tricresol formalin, formocresol, and CMCP), and calcium hydroxide showed a varying degrees of elimination of the bacteria. Tricresol formalin and formocresol decreased the bacterial count dramatically which coincides with the results of Simon et al (9) who reported the dramatic effectiveness of formocresol up to 7 days CMCP showed an inferior vapor action than the former two medicaments which may be due to its rapid dissipation after 24 hours as reported by Messer and Chen (10).

The bacterial count of the acetic acid group remained static during the 3 days between the first and second appointments. This stasis may be due to the long action of remnants of acetic acid left in the canal after drying in the first appointment, or due to a prolonged antibacterial effect on the bacteria, therefore delaying their growth.

There was an increase in the number of viable bacteria in the group of normal saline by 50% from that of the first appointment which agrees with Gutierrez (11) who indicated that normal saline has no antibacterial action and is not enough to eradicate all the bacteria in the root canal.

Calcium hydroxide’s elimination of bacteria was not as effective as the other medicaments, which complies with the results of Sjogren et al (12) who found that calcium hydroxide has a good antibacterial only in long periods as 1 week and more.

The mechanical debridement of the root canals decreased the bacterial count from one fifth to one
Clinical evaluation of the microflora present before debridement. These findings agree with the results of Bystrom and Sundqvist (8) who experienced a noticeable decrease in the number of bacteria were they found 8 out of 15 root canals to be sterile due to mechanical debridement but our study encountered a decrease in bacterial number but no negative cultures.

In the third appointment, 2% acetic acid, tricresol, formalin, and formocresol exhibited very low percentage of viable bacteria. CMCP was next with slightly more surviving bacteria. Calcium hydroxide showed more viable bacteria in the root canals which may be due to its slow diffusion through the smear layer and its poor antibacterial action (13).

Two percent acetic acid kept a stasis of the bacterial count which may be due to its removal of the smear layer, opening the dentinal tubules, and the residual acetic acid staying in the root canal. The formaldehyde containing compounds achieved the highest percentage of negative cultures, which is due to the strong antibacterial action of formaldehyde vapor as found by Simon et al (9). Acetic acid gained 90% negative cultures which is better than the results of Leonardo et al (3) who flooded the root canals with 2% chlorhexidine to gain 77.8% negative cultures. CMCP gained 70% negative cultures which agree with the findings of Stuart et al (14), who concluded that two thirds of the root canals medicated with CMCP were sterile.

Ca (OH)2 gained 60% negative cultures which is slightly lower than the results of Safavi et al (15), who stated that Ca (OH)2 gave a 74 % negative cultures in treated root canals. This difference may be due to the difference in the time period, where Ca (OH) 2 was kept in the root canals for 3 days in our study and for 7 days in Safavi’s study. Finally, normal saline exhibited 30% negative cultures.

REFERENCES