Clinical Efficacy of Treatment Procedures in Endodontic Infection Control and One Year Follow-Up of Periapical Healing

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Abstract

The objective was to evaluate the clinical efficacy of chemomechanical preparation of the root canals with sodium hypochlorite and interappointment medication with calcium hydroxide in the control of root canal infections and healing of periapical lesions. Fifty teeth diagnosed with chronic apical periodontitis were randomly allocated to one of three treatments: Single visit (SV group, n = 20), calcium hydroxide for one week (CH group n = 18), or leaving the canal empty but sealed for one week (EC group, n = 12). Microbiological samples were taken to monitor the infection during treatment. Periapical healing was controlled radiographically following the change in the periapical index at 52 wk and analyzed using one-way ANOVA. All cases showed microbiological growth in the beginning of the treatment. After mechanical preparation and irrigation with sodium hypochlorite in the first appointment, 20 to 33% of the cases showed growth. At the second appointment 33% of the cases in the CH group revealed bacteria, whereas the EC group showed remarkably more culture positive cases (67%). Sodium hypochlorite was effective also at the second appointment and only two teeth remained culture positive. Only minor differences in periapical healing were observed between the treatment groups. However, bacterial growth at the second appointment had a significant negative impact on healing of the periapical lesion (p < 0.01). The present study indicates good clinical efficacy of sodium hypochlorite irrigation in the control of root canal infection. Calcium hydroxide dressing between the appointments did not show the expected effect in disinfection the root canal system and treatment outcome, indicating the need to develop more efficient interappointment dressings.

Apical periodontitis is caused by root canal infection usually dominated by obligate anaerobes, and the number of cultivable species varies from two to eight (1). Recent molecular analyses of the microflora suggest a much higher number of microbial species (2). Control and elimination of the root canal infection is achieved by the combined action of several treatment procedures. During treatment, ecological conditions change remarkably in the root canal system contributing to the elimination of the microflora. Chemomechanical preparation aims at removing necrotic pulp tissue and infected dentine, and mechanical instrumentation augmented with antimicrobial irrigation kills the majority of the microorganisms from the root canal. However, some microorganisms may survive and therefore, an interappointment dressing, commonly calcium hydroxide, is often used to complete the disinfection of the root canal system before obturation.

Sodium hypochlorite is an effective disinfectant used for irrigation during mechanical preparation of the root canals. It has a wide antimicrobial spectrum and is potent also in low concentrations (3, 4). Calcium hydroxide is a commonly used root canal dressing. It has been found useful in treatment of immature roots for completion of root end formation, of root open teeth for the formation of an apical barrier, in traumatized teeth for the prevention or arrest of inflammatory root resorption, and of infected pulps with apical periodontitis (5). The antibacterial properties of calcium hydroxide are of primary importance, and several laboratory and clinical studies have testified to the efficacy of calcium hydroxide in the treatment of infected root canals (6–9). Its anti-microbial activity in the root canal is supposedly based on its high alkalinity and mechanical blocking of nutrients from the periapical area. Calcium hydroxide is effective on the majority of bacteria isolated from infected root canals (6, 10). However, the microflora of infected root canal is, occasionally, resistant against routine treatment procedures and medicaments. Microbiological investigations have shown that Enterococcus faeacalis and Candida albicans can often be isolated from such persistent infections (6, 11–13). Several factors may contribute to the survival of these microorganisms during the treatment. They may for example tolerate high alkalinity caused by calcium hydroxide or they may be capable to penetrate into dentinal tubules and thus avoid effective concentrations of therapeutic agents (10, 14). It may be seen as a problem that a medicament, in this case calcium hydroxide, indirectly may favor the growth of relatively resistant organisms, which may maintain the infection and be even more resistant to therapeutic efforts. The tooth may also be susceptible to reinfection through the temporary filling and dressing during the interim period.

Many in vitro studies have focused on the efficacy of different irrigants and interappointment dressing against microorganisms isolated from root canal infections. The aim of the present study was to evaluate the clinical efficacy of chemomechanical preparation with sodium hypochlorite and interappointment medication with calcium hydroxide in the control of the root canal infection. The hypothesis of the study was that using calcium hydroxide as an interappointment dressing would result in a higher percentage of root canals with no cultivable microorganisms in comparison to such root canals treated only with chemomechanical preparation using sodium hypochlorite but no interappointment dressing. Furthermore, better treatment outcome was expected for the teeth treated with calcium hydroxide interappointment dressing than for...
the teeth without the dressing or for the teeth subjected to treatment completed in one appointment.

**Materials and Methods**

Fifty teeth diagnosed with chronic apical periodontitis with per- apical index (PAI) score 3, 4, or 5 (15), and with a positive microbiological sample in the beginning of the treatment were included in the present study. Trope and co-workers analyzed previously radiological outcome of these cases after three different treatment strategies (16). In the present study, the case selection criteria were different, and therefore, the number of cases is smaller. This study focuses on the microbiological status at the time of root filling and its impact on radiological healing of apical periodontitis. The teeth were randomly subjected to one of three different treatment strategies. The root canal treatment was carried out as single visit treatment (single visit, SV group, n = 20), or using calcium hydroxide as root canal dressing between the first and second appointment (CH group, n = 18), or leaving the canal empty between the appointments (Empty canal, EC group, n = 12). The project was approved by the Committee on Investigations Involving Human Subjects at the University of North Carolina, School of Dentistry. All patients read and signed a consent form before initiation of the treat-

**Examination and Diagnosis**

The initial examination entailed registration of soft tissue status, percussion sensitivity, tooth mobility, coronal, and radicular restora-
tions present, presence of approximal contacts, antagonists, and pro-
thetic involvement, and marginal bone level. The pulpal diagnosis was
made based on anamnestic, X-ray examination using individual bite-
mount radiography, examination of pulp contents, and patient re-
sponse to initial instrumentation as described previously (7)

**Endodontic Treatment and Microbiological Sampling**

A rubber dam, clamp and ligature were used to establish the working
field. Preparative crown build-up was performed whenever neces-
sary for clamp and rubber dam retention. The access cavity was pre-
pared before or after the application of the rubber dam, but the dam was
always placed before entry into the pulp chamber. On completion of the
access cavity and before entry into the pulp chamber, preparations for
asepsis were carried out. The tooth, cavity, and one inch of the dam
surrounding the tooth were disinfected for 1 min by vigorous swabbing
with 0.12% chlorhexidine gluconate and thereafter only sterile instru-
ments were used. Microbiological control samples taken from the
working field after surface disinfection were uniformly negative.

Microbiological samples were taken twice during each appoint-
ment. After the initial access the first access sample (A1) was taken from
the pulp cavity and/or the pulp canal with one or several sterile paper
points. In some cases access into the canal space was eased by prior
insertion and withdrawal of a small size K-type file. Mechanical root
canal instrumentation was performed with stainless steel instruments
according to a standardized method as previously described (7). So-
dium hypochlorite (0.5%) was used as an irrigant during the prepara-
tion rinsing the canals thoroughly after each file size. When instrumen-
tation was complete, the canal was dried using sterile paper points. To
neutralize the antimicrobial action of sodium hypochlorite, an excess of
sodium thiosulphate was used to irrigate thoroughly the entire canal,
and the canal was dried again. A sterile reamer or file one size larger
than the last apical file was chosen, inserted to the full canal length,
turned clockwise 360 degrees and withdrawn. The apical 5 to 8 mm of
the tip of the instrument was cut off with a sterilized or flamed cable
cutter, making sure that the cut end fell into a vial with RTF [first post
irrigation sample (P11)] (17). Flamed cable cutter was controlled not
to cause contaminations during the sampling. At the second appoint-
ment, the root canals in the CH group were rinsed and neutralized with
0.5% citric acid and then with saline. The canals in the EC group were
rinsed with saline. The canal walls in both groups were vertically
worked on with a reamer the same size as the last used at the previous
appointment. The liquid in the canal was soaked up in paper points until
dry, and the points transferred aseptically to a vial with 1 ml RTF with
glass beads [second access sample (A2)]. A second post irrigation
sample (P2) was then taken with a reamer one size up as at the first
appointment.

**Laboratory Procedures**

The bacteriological specimens were processed within 2 h. They
were vortexed for 30 s and then transferred to an anaerobic chamber,
where all further processing took place. The samples were serially
diluted in RTF and cultivated anaerobically on sheep blood cell agar
and incubated at 37°C for 7 days. Representatives of observed colony types
were selected and pure cultures were made. The identification was done
by routine biochemical procedures designed for clinical purposes.
Therefore, some bacteria are not identified in detail.

**Radiological Follow-Up**

The periapical conditions were assessed radiographically using
the PAI-system (15). The radiological examination was done with indi-
vidually bite-mounted radiograph holders throughout the follow-up pe-
riod. Control radiographs were taken at 4, 12, 26, and 52 weeks after
the therapy and the PAI scores was recorded. Periapical healing, calcu-
lated as a reduction in PAI score from baseline, in different treatment
groups was compared. In addition, because of the aim to find associa-
tions between microbiological status at the time of root filling and peri-
apical healing, the teeth in groups CH and EC were pooled and then
subdivided in a group with bacteria present (BP group) and one with
bacteria absent (BA group) according to whether or not A2 sample was
positive for growth. The change of PAI was analyzed at 52 wk using
one-way ANOVA.

**Microbiology**

**First Appointment**

All three groups showed comparable proportions of growth pos-
itive samples taken during the first appointment. In the access sample
(A1) all cases included the study were growth positive. Post irrigation
samples (P1) in—the SV, CH, and EC groups showed growth percent-
ages of 20, 22, and 33%, respectively (Table 1).

**Second Appointment**

**CH group**

Six A2-samples (33%) showed growth. Chemomechanical prepa-
ration during the second appointment eliminated microorganisms effi-
ciently and no growth was detected in the P12 samples (Table 1).

**EC group**

The sampling taken in the beginning of the second appointment
(A2) showed growth in eight samples (67%). After irrigation with
sodium hypochlorite only two P12 samples (17%) showed growth (Table
1).

**Microorganisms**

Identification of the isolated microorganisms revealed a number of
species. The access sample in the first appointment (A1) showed
generally mixed infections dominated by anaerobic microorganisms.
Although the total number of growth positive cases decreased approx-
The bacteriological status at the appointment of root filling had a remarkable impact on the healing of the lesion. In the BA group the mean change of PAI at the 1-yr control was 1.45, whereas the BP group showed only 0.79 mean change of PAI. This difference was statistically significant ($p < 0.01$) (Fig. 2).

**Radiological Follow-Up**

The SV, CH, and EC groups showed only minor differences in the radiological healing of the periapical lesion. The mean change of PAI in each group at 1 yr control was $-1.45$, $-1.28$, and $-1.09$, respectively (Fig. 1).

The bacteriological status at the appointment of root filling had a remarkable impact on the healing of the lesion. In the BA group the mean change of PAI at the 1-yr control was $-1.53$, whereas the BP group showed only $-0.79$ mean change of PAI. This difference was statistically significant ($p < 0.01$) (Fig. 2).

**Discussion**

The effect of different treatment procedures on the control of root canal infection and its impact on the radiologically observed healing of the periapical lesion was studied. The access sample (A1) taken during the first appointment showed generally mixed cultures dominated by anaerobes. The microbiological status found is in accordance to the other studies on the cultivable microflora of root canal infections before treatment (1). Chemomechanical preparation decreased the percentage of growth positive samples (Pi1) to 20 to 33%. This indicates a good efficacy of mechanical instrumentation and sodium hypochlorite against the infective microflora within a short period of time, and is in accordance with previous studies (3, 4, 8, 18). Microbes that survived during the chemomechanical preparation may have avoided efficient concentrations by penetration into dentinal tubules or by biofilm formation on dentin, or because of inactivation of medicaments (19, 21).

The EC group showed growth in 67% of the samples taken in the beginning of the second appointment (A2). This is clearly more than the percentage of growth positive samples (Pi1) to 20 to 33%. This indicates a good efficacy of mechanical instrumentation and sodium hypochlorite against the infective microflora within a short period of time, and is in accordance with previous studies (3, 4, 8, 18). Microbes that survived during the chemomechanical preparation may have avoided efficient concentrations by penetration into dentinal tubules or by biofilm formation on dentin, or because of inactivation of medicaments (19, 21).

The EC group showed growth in 67% of the samples taken in the beginning of the second appointment (A2). This is clearly more than the percentage of growth positive samples (Pi1) to 20 to 33%. This indicates a good efficacy of mechanical instrumentation and sodium hypochlorite against the infective microflora within a short period of time, and is in accordance with previous studies (3, 4, 8, 18). Microbes that survived during the chemomechanical preparation may have avoided efficient concentrations by penetration into dentinal tubules or by biofilm formation on dentin, or because of inactivation of medicaments (19, 21).

**Figure 1.** Change of PAI in different treatment groups.

**Table 1.** Number of cases (%) with microbial growth in samples taken during first and second appointment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Access (A1) n (%)</th>
<th>Post irrigation (Pi1) n (%)</th>
<th>Access (A2) n (%)</th>
<th>Post irrigation (Pi2) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single visit</td>
<td>20 (100)</td>
<td>4 (20)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calcium hydroxide</td>
<td>18 (100)</td>
<td>4 (22)</td>
<td>6 (33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Empty canal</td>
<td>12 (100)</td>
<td>4 (33)</td>
<td>8 (67)</td>
<td>2 (17)</td>
</tr>
</tbody>
</table>

**Table 2.** Microbiological findings in growth-positive samples taken in the beginning of the treatment and after chemomechanical preparation during the first appointment.

<table>
<thead>
<tr>
<th>Species</th>
<th>A1, n (%)</th>
<th>Pi1, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive anaerobic rods</td>
<td>33 (66)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>Peptostreptococcus sp.</td>
<td>20 (40)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Gram-positive facultative rods</td>
<td>17 (34)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Gram-negative anaerobic rods</td>
<td>17 (34)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Veillonella sp.</td>
<td>13 (26)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Alpha-haemolytic</td>
<td>12 (24)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>6 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fusobacterium sp.</td>
<td>5 (10)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Prevotella sp.</td>
<td>4 (8)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>2 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Capnocytophaga sp.</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Leptotrichia buccalis</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Table 3.** Species isolated in the beginning of the second appointment (A2 sample) in Calcium hydroxide- and Empty canal–groups.

<table>
<thead>
<tr>
<th>Species</th>
<th>Calcium hydroxide, n (%)</th>
<th>Empty canal, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive facultative rods</td>
<td>3 (50)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Gram-negative anaerobic rods</td>
<td>3 (50)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Alpha-haemolytic Streptococcus sp.</td>
<td>2 (33)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Peptostreptococcus sp.</td>
<td>1 (17)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Veillonella sp.</td>
<td>1 (17)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Prevotella sp.</td>
<td>1 (17)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gram-positive anaerobic rods</td>
<td>0 (0)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Bacteroides sp.</td>
<td>0 (0)</td>
<td>1 (13)</td>
</tr>
</tbody>
</table>

Sp. = species.

A1 = Access sample 1.

Pi1 = Post irrigation sample 1.
It must be emphasized that an absence of bacteria before obturation resulted in the best treatment results. Therefore, rather than to conclude on the relevance of single vs. multiple visit protocols, one should continue to search for better antibacterial protocols to ensure that canals are predictably rendered bacteria-free before root filling.

References