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Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part II. *in vivo* studies

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The first part of this study reviewed the characteristics of calcium hydroxide ($Ca(OH)_2$) and summarized the results of *in vitro* studies related to its antimicrobial effects. The second part of this review covers *in vivo* studies including human clinical studies and animal studies. The use of $Ca(OH)_2$ as an intracanal medicament represented better histological results in animal studies. However, human clinical studies showed limited antimicrobial effects that microorganisms were reduced but not eliminated through the treatment, and that some species had resistance to $Ca(OH)_2$. Most of clinical outcome studies supported that there is no improvement in healing of periapical lesions when $Ca(OH)_2$ was applied between appointments. Further studies are required for the antimicrobial effects of $Ca(OH)_2$, and search for the ideal material and technique to completely clean infected root canals should be continued. (*Restor Dent Endod* 2015;40(2):97-103)

Key words: Antimicrobial effect; Calcium hydroxide; Endodontics; Intracanal medicament; Microorganism

Introduction

The first part of this study reviewed the characteristics of calcium hydroxide ($Ca(OH)_2$) and selected literatures dealing with *in vitro* antimicrobial effect of $Ca(OH)_2$ using antimicrobial susceptibility tests and infected dentin models. It was concluded that the antimicrobial effect of $Ca(OH)_2$ is related to the release of hydroxyl ions, and that $Ca(OH)_2$ had antimicrobial effect against common endodontic pathogens while it had limited effect against specific species such as *Enterococcus faecalis* (*E. faecalis*) or *Candida albicans* (*C. albicans*). The addition of other agents seems to be able to improve the antimicrobial effect of $Ca(OH)_2$.

The purpose of this article is to review the antimicrobial effect of $Ca(OH)_2$ as an intracanal medicament in root canal treatment. The second part of this review will cover *in vivo* studies including human clinical studies and animal studies. A PubMed search was performed to identify clinical studies that investigated the antimicrobial effect of $Ca(OH)_2$ from 1970 to 2013, and was limited to English-language papers. Studies that included $Ca(OH)_2$ as one of the comparative groups as well as the main subject were all reviewed. The articles were classified and analyzed according to their experimental methods.

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Review

In vivo studies

1. Animal studies (Table 1)

Stevens and Grossman used adult cats to determine the effectiveness of $Ca(OH)_2$ as an intracanal medicament.[1] Three of four canines were treated with $Ca(OH)_2$ solution or camphorated paramonochlorophenol (CMCP), and the remaining was used as control with no treatment. *E. faecalis* persisted in $Ca(OH)_2$ solution-treated canals for 3 weeks, whereas CMCP eliminated the infection after a single treatment.

Katebzadeh *et al*. histologically and radiographically compared the periapical healing of the infected roots obturated in one-step or with prior $Ca(OH)_2$ disinfection.[2,3] The result showed that 1 week $Ca(OH)_2$ disinfection before obturation results in significantly less periapical inflammation. Leonardo *et al*. performed histopathological evaluation of the repair of periapical tissues of dog's teeth with induced chronic periapical lesions after dressing with $Ca(OH)_2$ at different time periods.[4] The animals were sacrificed at 7, 15 and 30 days and the teeth specimens were examined with a light microscope. They concluded that more advanced repair was seen at 30 days compared to the other two periods. Tanomaru Filho *et al*. compared the periapical lesions of the dog teeth received immediate canal filling or 15 days $Ca(OH)_2$ dressing.[5] There was better histological repair in the groups with the $Ca(OH)_2$ dressing than the groups with immediate obturation. This small number of animal studies were the only ones that gave histopathological evidences.

De Rossi *et al*. evaluated healing of experimentally induced chronic apical lesions in dogs at 35, 75 and 120 days after instrumentation with or without $Ca(OH)_2$/chlorhexidine (CHX) paste intracanal dressing by standard radiographs.[6] Radiographs taken at 120 days showed that the treatment with intracanal medication resulted in a significant reduction in mean size of the apical lesions in comparison to single-session treatment.

2. Human clinical studies - bacterial culture method (Table 2)

Most clinical studies performed bacterial sampling from the canals for bacterial culture at three stages: S1, after initial access, to confirm that the canal is infected at the time of treatment; S2, after the cleaning and shaping procedure is complete, immediately before canal medication; S3, when the canal is re-accessed later and the medication has been removed. Some researchers additionally culture bacteria at certain days after cleaning the canal from medicaments (S4). S1 is essentially positive in 100% of the cases in teeth with periradicular lesion since they routinely have infected canals. Cleaning and shaping results in an extensive reduction in bacterial count

Table 1. Animal studies on the antimicrobial effect of $Ca(OH)_2$

Year	Researcher	Test method (animal)	Major ingredient	Period	Result
1983	Stevens & Grossman[1]	Culture (cat)	CH solution, slurry, Pulpdent, CMCP	21 day	CH solution: ineffective CH slurry, Pulpdent: limited effect
1999	Katebzadeh *et al*.[2]	Histopathology (dog)	CH	1 wk (sacrificed 6 mon)	CH: less inflammation
2000	Katebzadeh *et al*.[3]	Radiograph (dog)	CH	1 wk (x-ray 6 mon)	CH: fewer failed cases more improved cases
2002	Leonardo *et al*.[4]	Histopathology (dog)	CH + CMCP		30 day: Better results
2002	Tanomaru Filho *et al*.[5]	Histopathology (dog)	CH + CMCP	15 day (sacrificed 210 day)	Better results than immediate obturation
2005	De Rossi *et al*.[5]	Radiograph (dog)	CH + CHX	15 day (x-ray 30, 75 and 120 day)	120 day: Reduction of lesion size

CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; CHX, Chlorhexidine.
Pulpdent (Pulpdent Corp., Watertown, MA, USA).

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(above 99%).

In 1985, Byström *et al*. evaluated the bactericidal efficacy of $Ca(OH)_2$, camphorated phenol (CP), and CMCP as root canal dressings. The mechanical cleansing was completed at the first appointment and a sample was taken with paper points (S2).[7] Then the intracanal medicament was placed into the canal. At the second appointment, 1 month later for canals filled with $Ca(OH)_2$ paste, the intracanal dressing was removed and a sample was collected from the root canal (S3). After 2 to 4 days, the root canal was filled with saline and another bacteriological sample was taken (S4). Bacteria could not be recovered from any of these canals after they had been dressed with $Ca(OH)_2$ paste for 1 month. In samples taken 2 to 4 days after the dressing had been removed, bacteria were recovered from 1 of 35 root canals. Similar studies were performed with other researchers, and they also showed the antibacterial effect of $Ca(OH)_2$ paste used as a long-term intracanal dressing material.[8-11]

Several studies indicated that the treatment with intracanal medicaments could markedly affect the diversity and quantity of cultivable microorganisms in infected canals, with some groups of microorganisms being more resistant to treatment than others. Peters *et al*. evaluated microorganism in root canals with or without $Ca(OH)_2$ medication.[12] They said that although $Ca(OH)_2$ was placed in the prepared canals, the number of positive canals had increased in the period between visits. $Ca(OH)_2$ could not totally prevent regrowth of bacteria. Zerella *et al*. said that complete disinfection was not achieved but all cases that initially harbored *Enterococcus* species were successfully disinfected.[13] Chu *et al*. found that Gram-positive facultive anaerobic cocci tend to predominate than Gram-negative obligate anaerobic rods after treatment.[14] Oncag *et al*. said that CHX was more effective than $Ca(OH)_2$ alone against *E. faecalis*. In the study of Sinha *et al*., $Ca(OH)_2$ showed limited efficacy against facultative anaerobes and *Candida* species, but was effective against obligate anaerobes.[15,16] Molander *et al*. treated teeth with $Ca(OH)_2$ for 2 months and found that there was no increased antimicrobial effect of $Ca(OH)_2$ even if it was left for longer periods in the root canal.[17]

Some authors state that $Ca(OH)_2$ has little effect in reducing microorganisms. Manzur *et al*. found that bacterial growth and CFU counts decreased significantly from S1 to S2, but differences between S2 to S3 were not statistically significant for $Ca(OH)_2$, CHX, or combination of both.[18] Vianna *et al*. reported that after chemomechanical preparation, no improvement was achieved by 7 days of intracanal dressing.[19]

3. Human clinical studies - molecular method (Table 3)

In the middle of 2000s, a major shift had occurred in microbiology from studies based on culturing to ones that utilize molecular techniques. The reasons for this

Table 2. Human clinical studies on the antimicrobial effect of $Ca(OH)_2$ by bacterial culture

Year	Researcher	Major ingredient	Period	Protocol	Effect
1985	Byström *et al*.[7]	CH, CP, CMCP	1 mon	S2-S3-S4	+
1985	Safavi *et al*.[8]	CH, IKI		S2-S3	+
1991	Sjögren *et al*.[9]	CH	1 wk	S1-S2-S3-S4	+
1997	Barbosa *et al*.[10]	CH, CMCP, CHX	1 wk	S2-S3-S4	+
1999	Molander *et al*.[17]	CH	2 mon	S1-S2-S4	+/−
2000	Shuping *et al*.[11]	CH	7 - 200 day	S1-S2-S3	+
2002	Peters *et al*.[12]	CH	1 mon	S1-S2-S3	+/-
2005	Zerella *et al*.[13]	CH, CHX, CH+CHX	7 - 10 day	S1-S2-S3-S2-S3	+/−
2006	Chu *et al*.[14]	CH, Ledermix, Septomixine	1 wk	S1-S3	+/−
2006	Oncag *et al*.[15]	CH, CHX, CH+CHX	2 day	S1-S2-S3	+/−
2007	Manzur *et al*.[18]	CH, CHX, CH+CHX	1 wk	S1-S2-S3	−
2007	Vianna *et al*.[19]	CH, CHX, CH+CHX	1 wk	S1-S2-S3	−
2013	Sinha *et al*.[16]	CH, CHX, CH+CHX	1 wk	S1-S2-S3	+/−

+/−, The result showed a limited effect.
CH, Calcium hydroxide; CP, Camphorated phenol; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide; CHX, Chlorhexidine.

shift included the increase in sensitivity, accuracy, and efficiency of molecular techniques, as well as the realization that many microorganisms that populate the oral cavity may not have been cultivated. The most popular techniques were those based on polymerase chain reaction (PCR) amplification of the 16S or other ribosomal DNA sequences. Because of the low sensitivity of culture methods, a negative result does not imply that the canal has been rendered sterile. It could mean that bacterial numbers have reached a threshold that is undetectable by culture and that may be compatible with periradicular tissue healing. Molecular methods can detect far fewer cells than culture and have the potential to demonstrate the actual effectiveness of a given antibacterial protocol and accurately establish the number of bacterial cells that characterize the threshold below which a satisfactory outcome can still be achieved. However, as with any other method, molecular methods have limitations. Most assays are qualitative, and detect only a few target species and cannot detect unexpected species. Enzymes in the sample could suppress the amplifying reaction. Molecular methods cost higher than conventional culture methods. Lastly, they might detect dead microorganisms that we could not distinguish from living ones.[20]

Tang *et al*. evaluated the efficacy of $Ca(OH)_2$ or Septomixine (Septodont, St. Maur Des Fosses, France) in eliminating residual intracanal bacteria during interappointment using molecular methods.[21] The PCR results showed that 25 of 31 examined canals were positively detected with residual microorganisms after instrumentation, irrigation with saline, and 1 week medication with either $Ca(OH)_2$ or Septomixine. They suggested that the conventional 1 week medication might not effectively inhibit residual bacterial growth in all root canals during interappointment intervals. In the studies of Sakamoto *et al*. and Siqueira *et al*., significant reductions in bacterial counts were observed between S1 and S2, and S1 and S3.[22,23] However, no statistically significant difference was observed between S2 and S3 samples with regard to the number of cases yielding negative cultures or quantitative bacterial reduction.

De Souza *et al*. used checkboard DNA-DNA hybridization technique to examine the effects of $Ca(OH)_2$ therapy on the microorganisms.[24] Significant differences in the microbiota from baseline to post-therapy were found, and the results indicated that conventional therapy with $Ca(OH)_2$ results in the reduction of pathogenic species associated with pulp necrosis. However, it did not eliminate the whole spectrum of microorganisms. The studies of Siqueira *et al*. reported that 1 week use of $Ca(OH)_2$ mixed with CHX or CMCP significantly reduced the bacterial counts.[25,26] Rocas and Siqueira said that bacterial diversity was clearly reduced after chemomechanical preparation and after the supplemental effects of the intracanal medication.[27,28] Most taxa was completely eradicated, or at least reduced in levels. However, detectable levels of rRNA, which is highly likely to represent viable cells, were still observed in S2 and S3 samples. Ito *et al*. investigated the root canal microbiota of primary teeth with apical periodontitis and the *in vivo* antimicrobial effects of $Ca(OH)_2$/CHX paste using both bacterial culture and checkerboard DNA-DNA hybridization technique.[29] The results of both methods exhibited that the prevalence of samples that were positive for the presence of microorganisms did not change, although the overall number of bacteria was dramatically diminished compared with initial contamination. The recent study showed that the number of positive cases decreased between S2 and S3 samples.[30]

Table 3. Human clinical studies on the antimicrobial effect of $Ca(OH)_2$ by molecular methods

Year	Researcher	Major ingredient	Period	Protocol	Effect
2004	Tang *et al*.[21]	CH, Septomixine	1 wk	S1-S3	–
2005	de Souza *et al*.[24]	CH	2 wk	S1-S3	+/–
2007	Sakamoto *et al*.[22]	CH + CMCP	1 wk	S1-S2-S3	–
2007	Siqueira *et al*.[23]	CH	1 wk	S1-S2-S3	–
2007	Siqueira *et al*.[25]	CH + CMCP	1 wk	S1-S2-S3	+
2007	Siqueira *et al*.[26]	CH + CHX	1 wk	S1-S2-S3	+
2010	Rocas *et al*.[27]	CH + CMCP	1 wk	S1-S2-S3	+/–
2011	Ito *et al*.[29]	CH + CHX	2 wk	S1-S4	+/–
2011	Rocas *et al*.[28]	CH, CH + CMCP	1 wk	S1-S2-S3	+/–
2013	Paiva *et al*.[30]	CH + CHX	1 wk	S1-S2-S3	+/–

+/–, The result showed a limited effect.
CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; CHX, Chlorhexidine.

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Table 4. Clinical outcome studies on the use of $Ca(OH)_2$ as an intracanal medicaments

Year	Researcher	Test method	Interappointment period	Follow-up period	Result
1999	Trope *et al.*[34]	Radiograph	1 wk	1 yr	+
2000	Weiger *et al.*[35]	Radiograph	7 - 47 day	5 yr	–
2002	Peters and Wesselink[36]	Radiograph	4 wk	4.5 yr	–
2005	Waltimo *et al.*[37]	Radiograph	1 wk	1 yr	–
2007	Molander *et al.*[38]	Radiograph	1 wk	2 yr	–

+, The use of $Ca(OH)_2$ resulted better outcome; –, The results did not show significant difference.

4. Clinical outcome studies (Table 4)

One-visit root canal treatment has been considered ideal for the teeth with vital pulp.[31] On the other hand, root canal treatment for teeth with necrotic pulps associated with a periapical lesion remains controversial. Several studies have shown that it is impossible to eliminate whole microorganisms even after cleaning, shaping and irrigation with disinfectants.[7,12,32,33] Furthermore, a number of above clinical studies emphasizes that $Ca(OH)_2$ cannot completely reduce the remaining bacteria.

Trope *et al*. evaluated radiographic healing of teeth with apical periodontitis treated in one-visit or two-visit with or without $Ca(OH)_2$ as an intracanal medicament.[34] The Periapical Index (PAI) Scoring Method was used to compare differences in periapical status during one-year follow-up evaluation. It was shown that the use of $Ca(OH)_2$ before obturation resulted in 10% increase in healing rates.

On the other hand, several studies have concluded that one-visit treatment is equally effective or even more effective than multiple-visit treatment. Weiger *et al*. performed a prospective study to explore the influence of $Ca(OH)_2$ as an inter-appointment dressing on the healing of periapical lesions associated with pulpless teeth.[35] In both treatment groups, the success rate within an observation time of 5 years exceeded 90%. A statistically significant difference between the two groups was not detected. Peters and Wesselink found no significant differences in healing of periapical radiolucency between the teeth that were treated in one-visit and two-visits with $Ca(OH)_2$ for 4 weeks.[36] Waltimo *et al*. stated that $Ca(OH)_2$ dressing between the appointments did not show significant effect in treatment outcome.[37] In a randomized clinical trial, Molander *et al*. assessed 2 year clinical and radiographic outcomes of one- and two-visit root canal treatments and found similar healing results.[38]

In summary, there is still considerable controversy about the effect of the use of $Ca(OH)_2$ on the clinical outcome, while the majority of the studies supported that there was no significant difference in healing of periapical lesions between the treatment modalities.

Conclusions

To summarize the results of these *in vivo* studies, the use of $Ca(OH)_2$ as an intracanal medicament represented better histological results in animal studies. However, human clinical studies showed limited antimicrobial effects which microorganisms were reduced but not completely eliminated after treatments, and some species had resistance to $Ca(OH)_2$. The majority of clinical outcome studies supported that there was no improvement in healing of periapical lesions when $Ca(OH)_2$ was applied between appointments. It was concluded that one-visit treatment is as effective as two-visit treatment with interappointment intracanal medicaments. These may imply that there may be no correlation between the healing of endodontic lesions and the presence and absence of positive bacterial culture.

This series of articles reviewed currently available laboratory and clinical evidences addressing the antimicrobial effects of $Ca(OH)_2$ as an intracanal medicament. Although some studies have supported the effectiveness of $Ca(OH)_2$ as an intracanal medicament, others have questioned its efficacy or indicated that other agents should be mixed to improve its antimicrobial activity. It seems that the limitations of antimicrobial effect of $Ca(OH)_2$ has been suggested following the development of experimental methods. Further scientific investigations are required for better antibacterial protocols and sampling techniques to ensure that bacteria have been reliably eradicated prior to obturation, and it is necessary to assess the clinical outcomes related to intracanal medicaments. Also, further studies are required to assess the antimicrobial effect of $Ca(OH)_2$, and search for an ideal material and/or technique to completely clean infected root canals should be continued.

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