In vitro inhibition of bacteria from root canals of primary teeth by various dental materials

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Abstract

The primary tooth pulpectomy is a common clinical procedure. The choice of filling material is important to the success rate, but antibacterial properties of such materials against organisms known to inhabit infected primary root canals have not been well documented. This study compared the antibacterial effectiveness of 10 materials:

1. *Calcium hydroxide mixed with camphorated parachlorophenol ($Ca(OH)_2$+CPC)*
2. *Calcium hydroxide mixed with sterile water ($Ca(OH)_2$+ H_2O)*
3. *Zinc oxide mixed with CPC (ZnO+CPC)*
4. *Zinc oxide mixed with eugenol (ZOE)*
5. *ZOE mixed with formocresol (ZOE+FC)*
6. *Zinc oxide mixed with sterile water (ZnO+H_2O)*
7. *ZOE mixed with chlorhexidine dihydrochloride (ZOE+CHX)*
8. *Kri paste*
9. *Vitapex™ paste*
10. *Vaseline™ (control)*

These materials were compared against microbial specimens obtained from 13 infected primary teeth by using an agar diffusion assay. The results suggest that the materials could be divided into three categories. Category I, with the strongest antibacterial effect included ZnO+CPC, $Ca(OH)_2$+CPC, and ZOE+FC. Category II, with a medium antibacterial effect included ZOE+CHX, Kri, ZOE, and ZnO+H_2O. Category III, with no or minimal antibacterial effect included Vitapex, $Ca(OH)_2$+H_2O, and Vaseline. There were no significant differences within each category, but there were significant differences between the categories. The one exception was the antibacterial effect of ZOE+FC which was not significantly different from ZOE+CHX, Kri, or ZOE. (Pediatr Dent 17:351–55, 1995)

A pulpectomy is an acceptable treatment for saving infected primary teeth.[1] Clinical studies have reported success rates of 65–100% following this treatment.[2,3]

Success of endodontic treatment depends on the reduction or elimination of the infecting bacteria.[4] Among the ways to reduce or eliminate the infecting bacteria are: 1) adequate root canal debridement (instrumentation), 2) antibacterial irrigations, and 3) antibacterial filling materials — the focus of this investigation.

At least three dental products have been used routinely to fill root canals in primary teeth. Zinc oxide-eugenol (ZOE) has been the material of choice for many years. Although this agent showed antibacterial effects against pure cultures of bacteria in several studies,[5–7] combining with formocresol increased its antibacterial effect.[7,8] Another material, Kri paste, a mixture of iodoform, camphor, p-chlorophenol, and menthol also exerts a strong antimicrobial effect in vitro.[7,9,10] A third material, Vitapex, a mixture of iodoform and calcium hydroxide [$Ca(OH)_2$] demonstrated inhibitory activity against *Streptococcus mutans*, *Staphylococcus aureus*, and *Lactobacillus casei*. Cox et al.[8] found that zinc oxide alone could not inhibit *Echerichia coli*, *S. aureus*, or *Streptococcus viridans*, but ZOE inhibited *S. aureus* and *S. viridans*. The inclusion of zinc acetate as a setting accelerator, however, allowed ZOE to inhibit all three microorganisms. The inhibitory effect was further enhanced by adding formocresol. Wright et al.[17] reported Kri paste to be superior to ZOE in inhibiting *Streptococcus faecalis* in vitro.

Other calcium hydroxide-based products or materials containing chlorhexidine are used often in permanent teeth, but less so in primary teeth. One of these, K-20, a ZOE-based product containing chlorhexidine, demonstrated marked antibacterial effects in vitro.[12] Calcium hydroxide was shown by DiFiore et al.[16] to be noninhibitory against *Streptococcus sanguis* when mixed with water, but inhibitory when mixed with camphorated parachlorophenol (CPC). Other investigators found $Ca(OH)_2$ alone or as a cement (Dycal™, Caulk Division, Dentsply International Inc, Milford DE) to inhibit various species of bacteria in vitro.[9,14,15]

Previous investigations have demonstrated that root canal infections of primary teeth are usually

polymicrobial in nature.[18-20] One of these techniques (by Toyoshima et al.,[20] who employed anaerobic culture), showed a majority of the isolates to be obligate anaerobes with species of *Bacteroides, Eubacterium,* and anaerobic streptococci predominant. Most of the in vitro investigations of antibacterial activity of dental materials utilized pure cultures of facultative bacteria. None has tested anaerobic streptococci, eubacteria, *Bacteroides (Prevotella) intermedia,* or *Bacteroides (Prevotella) nodosus.*

The latter two species were predominant isolates in Toyoshima's study. If anaerobes comprise a majority of the bacteria in necrotic root canals of primary teeth, interpreting previous data where primarily facultative bacteria were used is therefore difficult.

The aim of our investigation was to determine the in vitro antibacterial effectiveness of several root canal filling materials against microbial specimens obtained directly from necrotic root canals of primary teeth using anaerobic methodology and agar plate growth inhibition.

Methods and materials

Dental materials

Nine dental materials and one control material (Vaseline™, Chesebrough Ponds USA, Greenwich, CT) were tested. These were: 1) calcium hydroxide (AMEND Drug & Chemical CO., Inc. Irrigation, NJ) mixed with camphorated parachlorophenol (U.S.P. Sultan Chemists, Inc. Englewood, NJ) ($Ca(OH)_2$+CPC), 2) calcium hydroxide mixed with sterile water ($Ca(OH)_2$+ H_2O), 3) zinc oxide (U.S.P. Sultan) mixed with CPC (ZnO+CPC), 4) zinc oxide mixed with eugenol (U.S.P. Sultan)(ZOE), 5) ZOE mixed with formocresol (Buckley's Formocresol, Sultan) (ZOE +FC), 6) zinc oxide mixed with sterile water (ZnO+ H_2O), 7) ZOE mixed with chlorhexidine dihydrochloride (ZOE+CHX) (Sigma Chemical Co. St. Louis, MO), 8) Kri paste (Pharmachemie AG, Zurich, Switzerland), 9) Vitapex paste (NEO Dental Chemical Products Co., LTD. Tokyo, Japan) and 10) Vaseline (control). Abbreviations and formulas for these agents are summarized in Table 1.

Microbial specimens

Single nonvital primary teeth, anterior or posterior, were extracted from 13 pediatric patients at the Pediatric Dentistry Postgraduate Clinic of the University of Maryland Dental School. Teeth employed in the microbiological experiments met the following criteria:

1. Contained at least one necrotic root canal
2. An abscess, fistula, or obvious radiolucency was present
3. Antibiotics were not received by the subject 4 weeks prior to sampling
4. Did not have resorbing roots or broken crowns.

Following extraction each tooth was transferred immediately to an anaerobic chamber (Coy Laboratory Products Inc, Ann Arbor, MI) in a vial containing prereduced transport fluid (RTF).[21] The bacterial contents of the root tips were then collected by filing the apical 3–4 mm of the root canals from the apical end of the roots with three sterile #15, 20, 25 endodontic K-files. The portions of all the files containing the specimen were cut with a sterile wire cutter into a vial containing 1 ml of RTF. Each specimen was assayed individually.

The bacteria were dispersed by sonication (Kontes Microultrasonic Cell Disrupter, Vineland, NJ) for 10 sec. Aliquots of the suspension containing the bacteria (100 µl) were spread on 90-mm-diameter petri dishes containing a nonselective medium, Brucella Agar (BBL, Cockeysville, MD) enriched with 3–5% sheep blood, hemin (5 µg/ml), and menadione (1 µg/ml), in order to prepare a lawn of the root canal bacteria. After the plate was dry, small wells (4 mm diameter and 3 mm deep) were made in the agar using a sterile amalgam carrier. Freshly mixed root canal filling materials were placed into the wells using a syringe or amalgam carrier. Control wells were filled with sterile Vaseline. Ten agar plates were required for each root canal specimen. Two filling materials were assayed on each plate. All plates were incubated at 37°C for 7 days by using an Anaerobic GasPak jar (B-D Microbiology Systems, Cockeysville, MD) and gas-generating envelopes (GasPak Plus, B-D Microbiology Systems) to achieve anaerobiosis. An anaerobic indicator strip (BBL) also was placed in the jars to monitor oxygen contamination of the environment.

After incubation, zones of inhibition (no growth of bacteria) were examined around wells containing filling materials. These appeared as clear, circular halos

TABLE 1. ABBREVIATIONS AND FORMULAS FOR TEST FILLING MATERIALS

CPC + $Ca(OH)_2$	Camphorated parachlorophenol: $Ca(OH)_2$ = 16 drops: 1 scoop = 0.16 cc: 0.17 g
CPC + ZnO	Camphorated parachlorophenol: ZnO = 8 drops: 1 scoop = 0.08 cc: 0.2 g
FC + ZOE	Formocresol: ZnO: Eugenol = 2 drops: 1 scoop: 6 drops = 0.02 cc: 0.2 g: 0.06 cc
CHX + ZOE	Chlorhexidine dihydrochloride: ZnO: Eugenol = 0.002 g: 0.198 g: 0.07 cc
Kri®	Commercial product
ZOE	Zinc oxide: Eugenol = 1 scoop: 7 drops = 0.2 g: 0.07 cc
ZnO + H_2O	Zinc oxide: Sterile water = 1 scoop: 7 drops = 0.2 g: 0.07 cc
$Ca(OH)_2$ + H_2O	Calcium hydroxide: Sterile water = 1 scoop: 10 drops = 0.17 g: 0.1 cc
Vitapex®	Commercial product
Vaseline®	Commercial product

surrounding the wells. Diameters of the zones were measured with a Boley gauge by one investigator. The mean of four measurements minus the 4-mm diameter of the well represented the inhibition value of the tested product. As several of the dental materials produced distinctive odors, an attempt to obscure the identity of the test agents was not made.

Table 2. Inhibition zone diameters of filling materials versus 13 root canal specimens

Agent	*Mean (mm)*	*SD*	*Range*
CPC + $Ca(OH)_2$	17.72	5.12	9.10–25.20
CPC + ZnO	15.96	6.10	7.50–29.50
FC + ZOE	12.76	5.42	3.30–21.90
CHX + ZOE	9.55	4.96	2.40–21.50
Kri™	9.50	5.18	0.00–19.20
ZOE	8.96	5.82	0.00–20.40
ZnO + H_2O	6.66	2.48	1.50–11.10
Vitapex™	0.99	2.43	0.00–6.80
$Ca(OH)_2$ + H_2O	0.30	1.08	0.00–3.90
Vaseline™	0.00	0.00	0.00–0.00

Statistical analysis

Data collected were analyzed by using SPSS for Windows 6.0 (SPSS Inc, Chicago, IL) on a personal computer. All data collected were first tested for normality. Logarithmic transformation was used to improve the normality. For a normally distributed dataset, a parametric test, analysis of variance with repeated measurement (ANOVA), was utilized to detect the statistical differences among materials tested; otherwise, the nonparametric Friedman test was used. Multiple comparisons were analyzed by using Tukey's HSD (honestly significant difference, $P < 0.05$) test.

Results

A total of 13 infected teeth (five anterior and eight posterior teeth) were extracted from 13 unrelated individuals and microbial specimens from them were processed for agar diffusion assay. All of these samples demonstrated polymicrobial infection. The descriptive statistics of zone data are shown in Table 2. In decreasing order of inhibitory activity, the test agents can be listed as follows: $Ca(OH)_2$+CPC, ZnO+CPC, ZOE+FC, ZOE+CHX, Kri, ZOE, ZnO+H_2O, Vitapex, $Ca(OH)_2$+H_2O, and Vaseline. As the data did not present a normal distribution, a nonparametric test (Friedman test) was used for data analysis. This revealed that zone sizes of all agents were significantly different (Chi-Square:94.77, $P < 0.001$). A Tukey HSD multiple comparisons test showed that the 10 test materials could be divided into three categories (Figure). Category I, the strong antibacterial effect group, included $Ca(OH)_2$+CPC, ZnO+CPC, and ZOE+FC. Category II, medium antibacterial effect group, included ZOE+CHX, Kri, ZOE, and ZnO+H_2O. Category III, no or minimal antibacterial effect group, included Vitapex, Ca(OH)2+H_2O, and Vaseline. There were no significant differences within each category, but there were significant differences between each category with the exception of FC+ZOE, which was not significantly greater than ZOE+CHX, Kri, or ZOE.

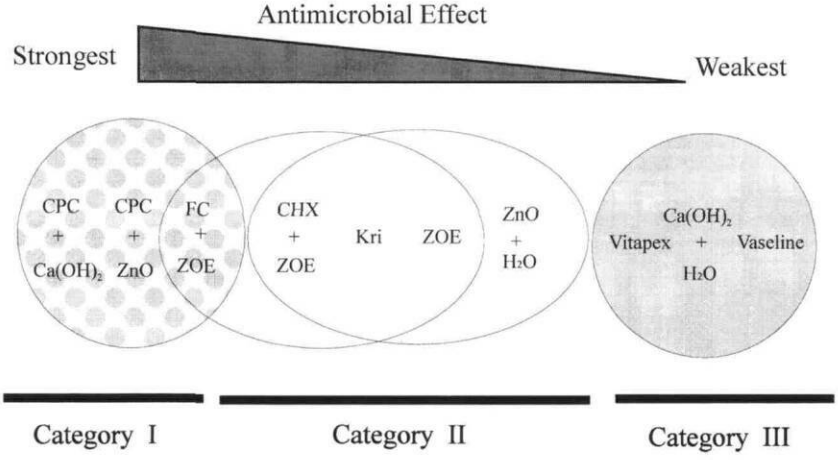

Figure. Inhibitory zones of test agents against mixed cultures of root canal bacteria. Results of multiple comparisons.

Discussion

The bacterial specimens in this study were collected from the root apex rather than the coronal portion to avoid potential contamination from cariogenic bacteria inhabiting the pulp chamber. Furthermore, oxygen contamination was reduced since high-speed drilling was not required.

An in vitro study can't simulate perfectly an in vivo situation, but it can control factors that an in vivo study can't, such as a quantitative evaluation of antibacterial activity by a wide variety of materials. As the in vitro method also required the filling material to diffuse into the agar, the net inhibitory effect was a combination of diffusion potential and antibacterial activity. The ability to diffuse into dentinal tubules is a desired characteristic of an antibacterial agent. Hobson found that microorganisms penetrated into the tubules of dentinal walls in root canals in 70% of extracted teeth with necrotic tissue.[22] Agents demonstrating the largest zones of inhibition in our study usually were the ones with the best diffusion capacity, but not always. $Ca(OH)_2$ demonstrated a diffusion zone, but did not inhibit bacteria growth. This finding agreed with Stevens and Grossman's that $Ca(OH)_2$ could not inhibit *S. faecalis* effectively, even though it achieved a large diffusion zone.[6]

To achieve a successful pulpectomy, good instrumentation, irrigation, intracanal medication, and use of an antiseptic filling material are important. If one can't achieve good instrumentation (i.e., on a tooth with root resorption) or intracanal medi-

cation (i.e., a one-appointment pulpectomy that doesn't provide for a residual antibacterial in the tooth), theoretically one must depend on antibacterial activity of the filling material.

The diversity of bacterial composition between root canals should be emphasized. This fact frequently determines clinical success or failure when a particular material is utilized. Such diversity was apparent in the relatively high standard deviations of inhibition zone diameters in our investigation (Table 2). More detailed studies of the nature of root canal microbiota and the response of specific bacteria to root canal filling materials should be pursued.

All the materials containing FC or CPC (category I materials, (Figure) exerted the strongest antibacterial effect against mixed cultures in our investigation. While results are difficult to compare due to many experimental differences, all previous pure culture studies also showed strong microbial inhibition when FC and CPC were tested. If these agents are used clinically, however, the benefit of antibacterial potential may be outweighed by the risk of tissue toxicity.[23–25]

This study found category II materials (ZOE + CHX, Kri, ZOE, and ZnO + H_2O) to consistently inhibit root canal microflora, but not to the extent of the category I materials (Figure). Again, while mixed culture results cannot be strictly compared to pure culture findings, most studies of the latter group showed ZOE, Kri, and K-20 (a ZOE plus chlorhexidine product),[12] to exert in vitro inhibition of a wide variety of mainly facultative or aerobic pure cultures. The anaerobes, *Bacteroides (Porphyromonas) gingivalis* and *Bacteroides (Porphyromonas) endodontalis*, were included.[5–12, 18] Our results showing antibacterial activity of ZnO may conflict with Cox et al.,[8] who found no activity of ZnO against *E. coli*, *S. aureus* or *S. viridans* in vitro. Although comparison difficulties exist, ZnO produced zones of inhibition with all 13 mixed culture specimens in our investigation and did not differ significantly from the other category II materials.

Additional conflicts exist with regard to category III materials ($Ca(OH)_2$ + H_2O, Vitapex, and Vaseline. Several reports claimed that $Ca(OH)_2$ was inhibitory to pure cultures of bacteria including *Bacteroides* species,[9, 14, 15] but our findings showed no inhibitory activity by $Ca(OH)_2$ + water. This is supported by DiFiore et al. who reported no inhibition of *S. sanguis* by $Ca(OH)_2$ and by Stevens and Grossman[6] who found it noninhibitory to *S. faecalis*.

The importance of antibacterial potential of a filling material to clinical success is debatable. Even teeth treated with Vitapex, a category III material with only slight antibacterial activity, achieved a clinical success rate of 86% according to one report.[2] If the category I materials are ruled out due to their potential toxicity in favor of category II materials, the latter offer reduced antibacterial activity, but perhaps less adverse tissue reactivity. Holan and Fuks[3] found that Kri paste was significantly superior to ZOE clinically. Differences in toxicity and resorbability of these agents may explain clinical differences, since our investigation could not differentiate them microbiologically. Meryon and Brook[27] and Wright[17] using tissue cultures found Kri paste to be toxic to mouse fibroblast cells. Wright reported ZOE to exhibit less toxicity in the same model. ZOE, on the other hand, resorbs slowly in vivo. Woods et al.[29] reported that ZOE showed delayed resorption clinically and caused transitory inflammation, but did not show cytotoxicity. Tronstad and Wennberg[30] found a slight cytotoxic effect of ZOE after 24 hr in vivo. Sadrian and Coll[28] found that retained ZOE did not cause significant clinical damage, even though it was resorbed slowly. In their study, 49.4% of cases retained ZOE after exfoliation of the treated primary tooth. Erausquin et al.[31] reported less favorable clinical outcomes with ZOE, stating that it was highly irritating to periapical tissues, caused necrosis of hard tissue, and showed a marked resistance to resorption. The authors also stated that if ZOE became mixed with tissue fluids, blood, or detritus, it was more rapidly adsorbed. Wright et al.[17] suggested eugenol as potentially cytotoxic since cytotoxicity of ZOE decreased after 1 or 7 days. The authors proposed that eugenol became bound after 24 hr when the cement set and was unavailable for tissue reactivity. Our recent investigation found antibacterial potential of ZOE and ZnO to be similar. Use of ZnO without eugenol as a pulpectomy filling material may be justified.

Conclusion

The 10 materials could be divided into three categories based on potency of antibacterial activity. The strong antibacterial effect group (category I) included $Ca(OH)_2$+CPC, ZnO+CPC, and ZOE+FC. The medium antibacterial group (category II) included ZOE+CHX, Kri, ZOE, and ZnO+H_2O. The no or minimal antibacterial group (category III) included Vitapex, $Ca(OH)_2$+ H_2O, and Vaseline. There were no significant differences within each category, but there were significant differences between each category, except for the antibacterial effect of ZOE + FC, which was not significantly different compared with ZOE+CHX, Kri, or ZOE.

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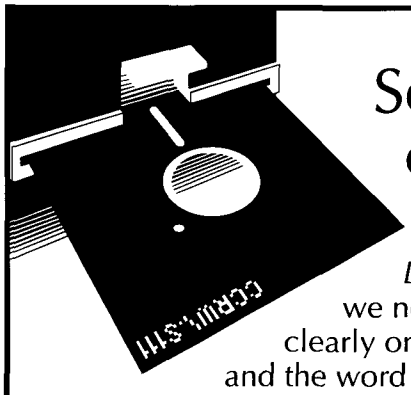

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