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The Antibacterial Effects of
Calcium Hydroxide Apexification Pastes on
Streptococcus sanguis

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ABSTRACT

Four calcium hydroxide based apexification pastes were tested for their antibacterial effects on *Streptococcus sanguis*. Their zones of growth inhibition on blood agar plates were measured at 2, 4, 6, and 8 days. Only the camphorated parachlorophenol and the meta-cresylacetate pastes showed zones of inhibition. Both of these zones of inhibition decreased with time, however, the zones of inhibition for the parachlorophenol paste decreased at a slower rate.

INTRODUCTION

Calcium hydroxide, used as an apexification paste, was introduced in the United States by Kaiser¹ and Frank.² They used calcium hydroxide mixed into a paste with camphorated parachlorophenol.^{1,2} Subsequently, much has been written about the use of this formula to induce closure of open apices.^{3,4}

Other vehicles for calcium hydroxide have been used for apexification pastes. These are metacresylacetate,⁵ methylcellulose,⁶ normal saline,^{7,8} and distilled water.⁹

Although camphorated parachlorophenol and metacresylacetate both have antibacterial properties, camphorated parachlorophenol is generally considered to be more antibacterial than metacresylacetate.¹⁰⁻¹²

Earlier bacteriological studies of infected endodontic systems showed a predominance of gram positive *Streptococcus viridans*,¹³ while more recent studies have shown them to be anerobic infections of indigenous bacteria.^{14,15} The cultivable bacteria from dental pyogenic infections have been shown to be a mixture of anerobic rods and cocci, and facultative streptococci.¹⁶ Representative bacterial species from these groups have been grown on blood agar medium to test the antibacterial effects on various medicaments.^{17,18}

At present, the antibacterial effects of the various calcium hydroxide pastes used for apexification have not been studied. Since the high pH of calcium hydroxide would make it antibacterial by itself, the question arises as to the necessity of using an antimicrobial or a neutral vehicle in the paste preparation.^{8,19}

The purpose of this study was to investigate the antibacterial effects of four calcium hydroxide pastes made individually with camphorated parachlorophenol, metacresylacetate, methyl cellulose and water.

MATERIALS AND METHODS

The testing technique used for this investigation is based on the standardized single disk method for antibiotic susceptibility.²⁰ A culture of *Streptococcus sanguis* (gram positive, facultative anerobe) was reconstituted from lyophilization and grown aerobically in trypticase soy liquid medium. Five prereduced blood agar culture plates (15 x 150mm) were seeded with this culture. A combination of the pour plate and cotton swab streaking methods were used to insure a complete and even distribution of the bacterial culture. Five penny cylinders (hollow stainless steel tubes 10mm long and 8mm in diameter) were placed in an even distribution over the seeded blood agar plates (Fig I). The four calcium hydroxide pastes to be tested were individually mixed by spatulation on a glass slab using a sterile technique. Two grams of powdered calcium hydroxide USP and 1cc of the appropriate liquid (camphorated parachlorophenol, metacresylacetate, or sterile distilled water) was mixed to produce a thick paste consistent with that used clinically. Calcium hydroxide and methycellulose (Pulpdent) was used directly out of its syringe dispenser. A half gram of each paste was condensed into a penny cylinder in direct contact with the blood agar surface of each plate.

The open ends of the penny cylinders were sealed with Cavit. The fifth empty penny cylinder, with its open end sealed with Cavit, was used as a control. The five seeded blood agar plates with the test medicaments on them were incubated anerobically using disposable gas pac generators. The diameters of the zones of bacterial growth inhibition were measured with a millimeter caliper at 2, 4, 6, and 8 days.

RESULTS

There were no zones of growth inhibition for the calcium hydroxide and water paste, the calcium hydroxide and methycellulose, or for the control. There were zones of growth inhibition for the calcium hydroxide and camphorated parachlorophenol paste, and for the calcium hydroxide and metacresylacetate paste over all four time periods.

The measurement data, paired differences, averages, paired average differences, standard deviations and paired t-test results are shown in Table I.

The average zone diameters decreased for both the camphorated parachlorophenol and metacresylacetate pastes but the average zone diameter rate of decrease for camphorated parachlorophenol was less (Fig II).

The difference between their effects at each time interval was shown by t-test to be statistically significant (Table I).

The paired average differences of the diameters of the zones of

inhibition decreased at each time interval (table I)

The Pearson's r between time and decrease in zone of inhibition for the metacresylacetate paste was -0.6198 and the camphorated para-chlorophenol paste was -0.3005 . This difference is significant at the 0.01 level of significance.

The regression coefficients (metacresylacetate paste $= -1.47$, camphorated parachlorophenol paste $= -0.644$) of the decrease in zone diameter over time between the two pastes was also statistically different ($p < 0.01$).

DISCUSSION

The size of the zone of bacterial growth inhibition does not necessarily reflect the strength of the antibacterial agent. The zone size may be influenced by the molecular size of the chemical and its diffusion constant. An agent which diffuses more easily will give a larger zone. The fact that there was no inhibition zone for the water paste and Pulpdent may just mean that they did not adequately diffuse through the medium. Both camphorated para-chlorophenol and metacresylacetate have been shown to easily diffuse through blood agar medium.²¹

In comparing the results obtained with the camphorated para-chlorophenol paste and the metacresylacetate paste, two observations require some explanation. First, the likely explanation for the larger zones obtained with the metacresylacetate paste was that more free metacresylacetate was available for diffusion and not

that it was more antibacterial. Second, the slower rate of reduction in the size of the zones obtained with the camphorated parachlorophenol paste indicates that either it maintains its antibacterial effect longer or that camphorated parachlorophenol is being released more slowly and therefore it is available longer to cause its effect.

Since the periapical tissues of teeth with infected root canals are generally free of bacteria,^{22,23} the important antibacterial effect is within the root canal system and not beyond the apical foramen. Diffusion may be an advantage in the micro-environment of the endodontic system where penetration through the dentinal tubules may expand antibacterial efficacy.^{24,25}

In the clinical situation, the calcium hydroxide camphorated parachlorophenol paste sets in a cement-like manner,² while the calcium hydroxide metacresylacetate paste remains unset.⁵ This may explain the greater availability of metacresylacetate to act initially while the camphorated parachlorophenol may be released more slowly and therefore may be available over a longer period of time. The slow release of camphorated parachlorophenol may allow for a minimal tissue irritational effect with a long term antibacterial effect. This may partially explain the excellent apical response reported with this mixture.^{2,4}

Streptococcus sanguis was chosen as a general test organism for this study because it is an indigenous oral facultative anerobic microorganism that is conveniently obtained and easily

grown in the laboratory. This study evaluates the relative effects on the microorganism of calcium hydroxide mixed with various liquids. Camphorated parachlorophenol and metacresylacetate have been shown to cause consistent antimicrobial results irrespective of the type of test organisms utilized.^{11,18} Therefore, for this study, it appeared that the use of the one microorganism would give the information desired.

SUMMARY AND CONCLUSIONS

Four apexification pastes (calcium hydroxide and camphorated parachlorophenol, calcium hydroxide and metacresylacetate, calcium hydroxide and methylcellulose (Pulpdent), and calcium hydroxide and distilled water) were tested for their antibacterial effects on *Streptococcus sanguis*, by measuring their zones of growth inhibition on blood agar plates over time periods.

1. Calcium hydroxide with distilled water and Pulpdent both showed no growth inhibition, whereas calcium hydroxide with camphorated parachlorophenol and calcium hydroxide with metacresylacetate both showed growth inhibition.

2. The inhibitory zones for both the camphorated parachlorophenol and metacresylacetate paste decreased with time, but the camphorated parachlorophenol paste decreased at a slower rate.

3. The camphorated parachlorophenol and metacresylacetate in calcium hydroxide pastes are capable of diffusion and thus expanding the area of antibacterial effect.

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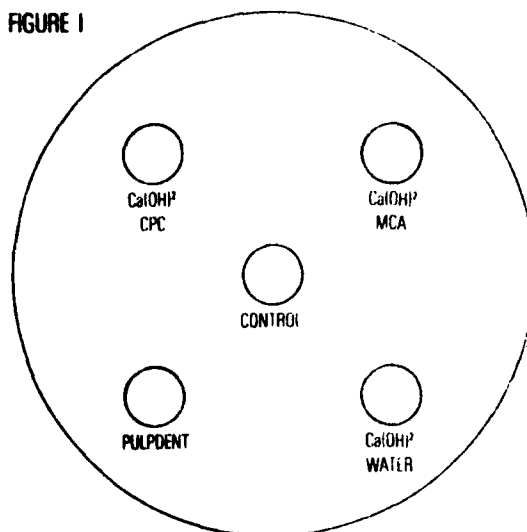
TABLE I

Days	Agent	Diameter of the Zones in mm					Average	SD	Paired t-Test
		1	2	3	4	5			
2	MCA	43.3	38.3	42.8	40.7	35.3	40.08	3.32	<.001
2	CPC	36.8	31.1	28.9	30.2	25.8	30.56	4.06	
Paired Difference		6.5	7.2	13.9	10.5	9.5	9.52		
4	MCA	39.5	36.4	40.4	38.7	28.9	36.78	4.65	<.01
4	CPC	34.6	30.9	27.6	29.1	21.5	28.74	4.82	
Paired Difference		4.9	5.5	12.8	9.6	7.4	8.04		
6	MCA	37.2	34.5	37.6	34.6	24.6	33.70	5.28	<.01
6	CPC	33.8	29.8	26.9	27.5	18.7	27.34	5.84	
Paired Difference		3.4	4.7	10.7	7.1	5.9	6.36		
8	MCA	35.1	32.4	33.9	32.7	22.4	31.30	5.09	<.001
8	CPC	33.2	29.2	26.5	26.9	18.1	26.78	5.53	
Paired Difference		1.9	3.2	7.4	5.8	4.3	4.52		

MCA = Metacresylacetate

CPC = Camphorated Parachlorophenol

FIGURE I



THE PLACEMENT PATTERN OF PENNY CYLINDERS
ON THE 15 x 150 mm BLOOD AGAR PLATES

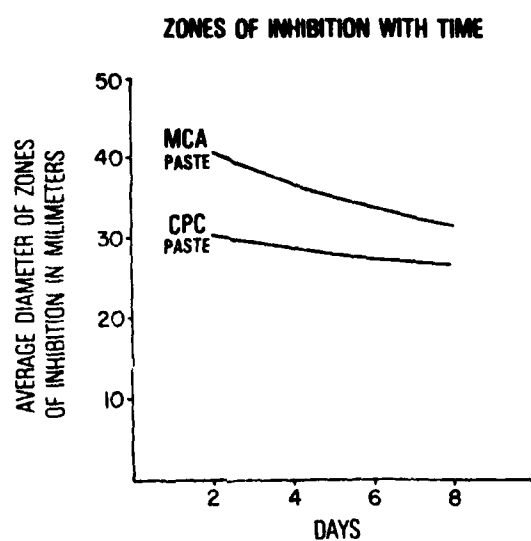


FIGURE II
GRAPH OF THE AVERAGE DIAMETER OF ZONES OF INHIBITION
AT EACH TIME INTERVAL FOR MCA AND CPC

MCA - METACRESYLACETATE
CPC - CAMPHORATED PARACHOLOPHENOL