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The effects of dyadic combinations of endodontic medicaments on microbial growth inhibition

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Abstract

In recent years dyadic combinations of endodontic medicaments have been used increasingly in clinical pediatric dentistry with little regard to the possibility of pharmacological antagonism of the components. In this investigation, a microbial growth inhibition assay was used to determine changes in antimicrobial activity in dyadic mixtures of endodontic medicaments. The combinations assayed were Ledermix® (corticosteroid-antibiotic) and Calyxl® (calcium hydroxide), Ledermix and Kri® (iodoform), Kri and Calyxl, and formocresol and eugenol. All these compounds have antibacterial activity when used individually. In the dyadic combinations assayed, results showed that adding calcium hydroxide to another antibiotic preparation has deleterious effects on growth inhibition, and combining any two antimicrobial medicaments produces no additive or synergistic effects. It is concluded that it may not be clinically advantageous to use endodontic medicaments in the dyadic combinations shown in this investigation.

Introduction

An important property of endodontic medicaments is antimicrobial activity, which is required to prevent or control existing pulp infection. The commonly used medicaments for endodontic treatment of primary teeth such as calcium hydroxide, zinc oxide-eugenol, formocresol, iodoform, and the corticosteroid-antibiotic pastes all have been shown to have varying degrees of antimicrobial activity (Treanor and Goldman 1972; Brilliant et al. 1974; Verco 1985).

Recently there have been reports of dyadic combinations of some pulp medicaments being used, although the efficacies of these combinations have not been established fully and their uses have not been recommended routinely in current pediatric text books (Stewart et al. 1982; McDonald and Avery 1987; Wei 1989). The reason usually given for mixing the medicaments is a putative advantage gained by obtaining the combined benefits of individual medicaments. Several investigators have suggested using corticosteroid-antibiotic pastes combined with calcium hydroxide to produce final mixtures with putative antimicrobial activity and osteogenic potential (Heithersay 1977; Abbott et al. 1989). The 50–50 mixture of Ledermix® (corticosteroid-antibiotic paste — Lederle Pharmaceuticals, Wolfrathausen, West Germany) with calcium hydroxide has been recommended as a pulp capping and pulpotomy agent (Schroeder 1972; 1981) as well as an intracanal dressing in cases of incomplete root canal formation, perforation, and tooth resorption (Heithersay 1977; 1984; 1985; Schroeder 1981).

Also, Craig et al. (1987) advocated using a combination of iodoform and antibiotic-corticosteroid mixture for endodontic management of abscessed primary teeth. In addition, several Japanese investigators suggested a combination of iodoform and calcium hydroxide pastes as root filling material for primary teeth (Fuchino 1980), and as a dressing to induce apexification for immature permanent teeth (Fujii 1984; Matsuzaki et al. 1987). A commercially available iodoform-calcium hydroxide paste (Vitapex® — Neo Dental Products, Tokyo) is now used widely in Japan (G. Goto, personal communication, 1989). Furthermore, the 50–50 mix of eugenol and formocresol has long been used as a pulp dressing after formocresol pulpotomies (Morawa et al. 1975). However, in the clinical use of these mixtures, very little consideration has been given to the possibility of pharmacological inactivation of the important properties of these medicaments when they are combined.

The aim of this study was to examine the changes in antimicrobial properties of endodontic medicaments commonly used in pediatric dentistry when they are mixed in dyadic combinations with other medicaments.

Materials and Methods

Endodontic Medicaments

The following proprietary brands of endodontic medicaments were used:

1. Ledermix paste, which contains 1% triamcinolone and 3% demethylchlortetracycline.

2. Calyxl® paste (Otto and Co, Frankfurt, W. Germany) which contains calcium hydroxide in physiological saline.

3. Formocresol (Creighton Pharmaceuticals, Sydney, Australia) which contains 19% formalin and 35% cresol.

4. Eugenol (David Craig and Co., Rocklea, Queensland, Australia) which contains 100% oil of cloves.

5. Kri® paste (Pharmachemie AG, Zurich, Switzerland) which contains 80.8% iodoform paste, 2.1% p-chlorophenolum, 4.8% camphor, and 1.2% mentholum.

The endodontic medicaments were used in the following dyadic combinations, which are often employed clinically: Ledermix and Calyxl, Ledermix and Kri, Kri and Calyxl, formocresol and eugenol. In each assay the concentrations of each component in a dyadic mixture varied from 75 to 25 per cent volume. Original medicament concentrations of 100% were included for direct comparisons.

The dyadic combinations were mixed thoroughly using an agitator (Super-mixer — Lab-line Instruments, Melrose Park, IL) for 2 min, then loaded into disposable tuberculin syringes to dispense the medicaments into microbial culture plates.

Microorganisms

The bacteria used in this investigation were two organisms commonly isolated from dental abscesses (Sabiston et al. 1976; Burnett and Schuster 1978): *Streptococcus sanguis* (UQM 2263) and *Staphylococcus aureus* (ATCC 25923). These were donated by the Department of Microbiology, University of Queensland and the Department of Microbiology, Mater Public Hospitals, South Brisbane, respectively. The bacteria were grown in blood agar and purity of cultures was established by Gram staining and colony characteristics (Seow et al. 1987). The bacteria were harvested into sterile test tubes containing normal saline, and the concentration of bacteria adjusted to 6×10^8/ml.

Microbial Culture Plates

The agar diffusion system first used by Nathan et al. 1978 to determine antimicrobial activity of various burn creams was employed. Petri dishes 90 mm in diameter were filled with 15 ml of Muller-Hinton agar, which is protein-free and one of the standard bacterial media used for investigations of antimicrobial sensitivity testing (Muller and Hinton 1941). In each agar plate, five holes, 5 mm in diameter were formed in the agar by removing plugs cut with a stainless steel borer. The holes were spaced approximately 30 mm apart and 20 mm from the outer edge.

All procedures were performed under sterile conditions. Each hole was filled with 0.1 ml of medicament dispensed from tuberculin syringes.

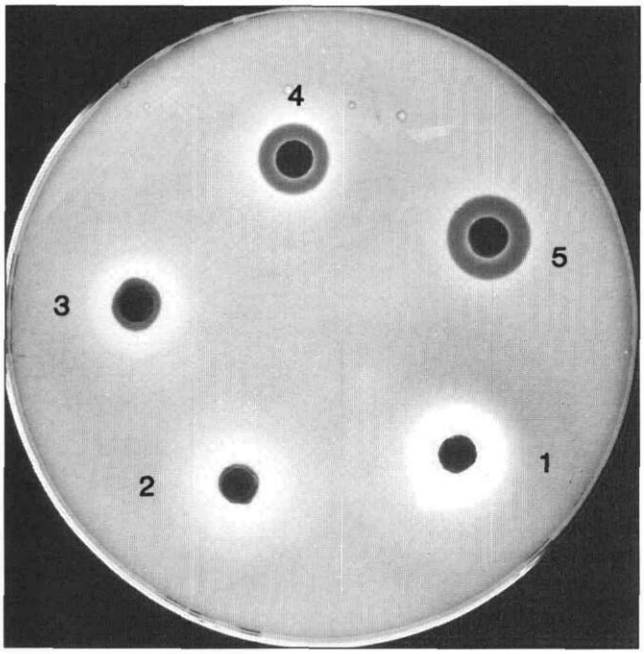

Fig 1. A microbial culture plate with wells containing dyadic combinations of Kri and Ledermix pastes. Well No. 1 contained 100% Kri. well No. 2 had 75% Kri and 25% Ledermix, well No. 3 had 50% Kri and 50% Ledermix, well No. 4 had 25% Kri and 75% Ledermix and well No. 5 had 100% Ledermix. In all wells, a clear zone of growth inhibition was observed. In addition, a dark zone was observed around wells 3–5. This was due to diffusion of Ledermix components through the agar. The bacteria cultured on this plate was *S. sanguis*.

When all the wells had been loaded with medicaments, 3 ml aliquots of bacterial suspensions were added to 3 ml of melted Muller-Hinton agar at 45°C. The bacteria suspensions in agar were mixed thoroughly and poured evenly over the surface of each agar plate.

The culture plates were inverted and incubated at 37°C for 24 hr. At the end of incubation, bacterial growth was confluent on the agar surface except at areas of growth inhibition. Fig 1 shows a culture plate at completion of the experiment.

In the evaluation of the test plates, a clear area around a test well indicated the presence of growth inhibition of the bacteria (Nathan et al. 1978; Holder 1981). The diameters of each area of growth inhibition were measured by using a millimeter ruler and by viewing the bottom of the agar plate. All measurements were performed in triplicate.

For each dyadic combination, four experiments were performed using the two bacteria.

Statistical Analysis

The Chi-square test and ANOVA as appropriate were used for statistical analysis of the data.

Results

Combinations of Ledermix and Calyxl

Table 1 shows the effects of combining Ledermix and Calyxl on microbial growth inhibition. As the table indicates, 100% Ledermix showed a zone of complete growth inhibition of mean diameter 10.5 ± 0.5 mm in the case of Strep sanguis and 22.3 + 2.0 mm in the case of Staph aureus. In contrast, 100% Calyxl resulted in a zone of only partial growth inhibition in both bacteria (12.1 ± 1.0 mm for *S. sanguis* and 13.1 ± 1.0 for *S. aureus*).

When Calyxl was added to Ledermix, the complete growth inhibition observed with 100% Ledermix was not present (Table 1). Instead it was replaced with a zone of only partial inhibition that increased as the concentration of Calyxl increased from 25 to 75%. These changes were statistically significant ($P < 0.01$).

Effect of Dilution of Ledermix

To determine if the effects observed in Table 1 were due to Ledermix being diluted, the experiments were repeated with the medicament combined with sterile normal saline. The results as shown in Table 2 indicated that diluting Ledermix to 25% of its original volume did not significantly affect its antimicrobial activity in this assay system ($P > 0.1$).

Similar observations were noted when Calyxl was diluted to 25% of its original volume (Table 3). No significant loss of antimicrobial activity was observed at all degrees of dilution of Calyxl.

Combinations of Ledermix and Kri

The effects of combining Ledermix and Kri are shown in Table 4. As this table shows, both 100% Ledermix and 100% Kri individually gave comparable zone(s) of growth inhibition of both bacteria (11.3 ± 0.2 for Ledermix and 11.9 ± 1.3 for Kri in the case of *S. sanguis* and 23.5 ± 0.9 for Ledermix and 24.1 ± 1.1 for Kri in the case of *S. aureus*).

It is interesting that similar values of inhibition were obtained at all dyadic combinations of Ledermix and Kri (Table 4), indicating that while no loss of antimicrobial activity occurred, there was also no synergism or additive antimicrobial activity when both medicaments were combined.

Combination of Kri and Calyxl

The effects of dyadic combinations of Kri and Calyxl on microbial growth inhibition are shown in Table 5. At 100% Kri, complete microbial growth inhibition was again observed (14.0 ± 0.6 mm for *S. sanguis* and 23.0 ± 0.8 for *S. aureus*). In contrast, 100% Calyxl produced only partial growth inhibition (10.7 ± 0.7 for *S. sanguis* and 10.6 ± 0.7 for *S. aureus*).

A dyadic combination of 75% Kri and 25% Calyxl produced significant loss of antimicrobial activity compared with 100% Kri (Table 5). In this mixture the zone of inhibition for *S. sanguis* was reduced from 14.0 ± 0.6 mm to 11.5 ± 0.9 mm, a loss of 17.8% growth inhibition ($P < 0.01$). In the case of *S. aureus*, the decrease was even more severe, from 23.0 ± 0.8 mm to 10.3 ± 0.5 mm, a loss of activity of 55.2% ($P < 0.001$).

At the dyadic combination of 50% Kri and 50% Calyxl, there was further loss of antimicrobial activity

TABLE 1. Effects of Dyadic Combinations of Ledermix and Calyxl on Inhibition of Microbial Growth

Dyadic Combination (% vol)		*Growth Inhibition mm (mean ± SD)*			
Ledermix	*Calxyl*	**Partial*		*Complete*	
		Ss	*Sa*	*Ss*	*Sa*
100	0	0	0	10.5 ± 0.5	22.3 ± 2.0
75	25	6.5 ± 0.4	9.0 ± 0.6	0	0
50	50	9.5 0.4	10.8 0.5	0	0
25	75	10.1 0.4	12.1 0.2	0	0
0	100	12.1 ± 1.0	13.1 ± 1.0	0	0

* The differences in partial growth inhibition using varying proportions of Ledermix and Calxyl are statistically significant, $P < 0.01$
Ss: *Strep. sanguis*
Sa: *Staph. aureus*

TABLE 2. Effects of Dilution of Ledermix Paste on Inhibition of Microbial Growth

Conc. of Ledermix (% vol)	*Growth Inhibition mm (mean ± SD)*	
	Strep. sanguis	*Staph. aureus*
100	10.5 ± 0.5	22.8 ± 1.9
75	10.0 0.5	22.2 1.5
50	9.6 0.4	23.0 1.6
25	9.5 ± 0.4	22.3 ± 1.7

For each bacteria, experiments were conducted in duplicate and three measurements were obtained from each experiment for each concentration of Ledermix. Hence the mean values shown above are the result of six separate measurements.
For each bacteria, the results at various concentrations were not statistically significant, $P > 0.1$
* The growth inhibition observed with Ledermix was complete growth inhibition.

TABLE 3. Effects of Dilution of Calyxl on Inhibition of Microbial Growth

Conc. of Calyxl (% vol)	*Growth Inhibition* mm (mean ± SD)*	
	Strep. sanguis	*Staph. aureus*
100	12.3 ± 0.9	13.1 ± 1.0
75	12.9 0.9	14.0 0.8
50	13.0 1.2	14.2 0.8
25	13.1 ± 0.8	12.9 ± 1.1

* The growth inhibition observed with Calyxl was partial inhibition.
For each bacteria, the results at various concentrations of medicament were not statistically significant, $P > 0.1$

TABLE 4. Effects of Dyadic Mixtures of Ledermix and Kri on Inhibition of Microbial Growth

Conc. of Medicament (% vol)		*Growth Inhibition mm (mean ± SD)*	
Ledermix	*Kri*	*Strep. sanguis*	*Staph. aureus*
100	0	11.3 ± 0.2	23.5 ± 0.9
75	25	10.6 0.4	24.5 0.9
50	50	10.9 0.6	23.8 1.0
25	75	10.8 0.7	23.6 1.1
0	100	11.9 ± 1.3	24.1 ± 1.1

* The growth inhibition detected at all concentrations of medicaments studied was complete growth inhibition.
The differences in results obtained at various medicament concentrations are not statistically significant, $P > 0.1$

TABLE 5. Effects of Dyadic Combinations of Kri and Calyxl on Microbial Growth Inhibition

Dyadic Combination (% vol)		*Growth Inhibition mm (mean ± SD)*			
Kri	*Calxyl*	*Partial*		**Complete*	
		Ss	*Sa*	*Ss*	*Sa*
100	0	0	0	14.0 ± 0.6	23.0 ± 0.8
75	25	0	0	11.5 ± 0.9	10.3 ± 0.5
50	50	0	0	9.0 ± 0.8	9.6 ± 0.5
25	75	9.5 ± 0.6	10.1 ± 0.7	0	0
0	100	10.7 ± 0.7	10.6 ± 0.7	0	0

Ss = *Strep. sanguis*
Sa = *Staph. aureus*
* The differences in complete growth inhibition between differing concentrations of Kri and Calyxl are statistically significant, $P < 0.01$

TABLE 6. Effects of Dyadic Combinations of Eugenol and Formocresol on Inhibition of Microbial Growth

Dyadic Combination (% vol)		**Growth Inhibition mm (mean ± SD)*	
Eugenol	*Formocresol*	*Strep. sanguis*	*Staph. aureus*
100	0	10.6 ± 0.9*	9.0 ± 0.8*
75	25	24.6 1.2	25.3 0.9
50	50	25.0 1.0	26.1 0.9
25	75	26.3 0.9	26.5 0.8
0	100	25.7 ± 1.1	27.1 ± 0.9

The differences in results between eugenol 100% and formocresol 100% are statistically significant, $P < 0.001$ for both bacteria.
* The growth inhibition observed with eugenol and formocresol at all concentrations are complete growth inhibitions.

with the zone of complete growth inhibition falling to 9.0 ± 0.8 mm (loss of activity of 35.7%, $P < 0.001$) in the case of *S. sanguis* and 9.6 ± 0.5 mm (loss of activity of 58.2%, $P < 0.001$) in the case of *S. aureus*.

When the volume percent of Calyxl increased further to 75%, the zones of complete growth inhibition were not present (Table 5). Instead a zone of partial growth inhibition appeared which was comparable in diameter to that of 100% Calyxl. This was observed for both bacteria.

Combinations of Eugenol and Formocresol

The effects of combining eugenol and formocresol are shown in Table 6. As the table shows, 100% eugenol produced significantly less microbial growth inhibition compared to 100% formocresol (10.6 ± 0.9 mm vs 25.7 ± 0.9 mm in the case of *S. aureus* and 9.0 ± 0.8 vs 27.1 ± 0.9 in the case of *S. sanguis*). Table 5 also shows that the addition of 25% formocresol to eugenol is sufficient to increase the antimicrobial effects to that comparable with 100% formocresol alone.

Discussion

Clinically acceptable endodontic medicaments should show antimicrobial activity and encourage healing of the pulp and periodontal tissues. However many currently available medicaments are far from ideal and show little therapeutic potential on the inflamed pulp (Schroeder and Granath 1971; Magnusson 1978). In addition, eugenol, formocresol, and calcium hydroxide also have been shown to possess inflammatory potential individually (Seow and Thong 1986).

The antibacterial properties of the endodontic medicaments used in the present study are already well established (Treanor and Goldman 1972; Rifkin 1980; Verco 1985) and probably contribute significantly to the clinical success of endodontic therapy by inhibiting residual bacteria not removed by mechanical debridement (Goerig and Camp 1983). In endodontics of primary teeth, the antimicrobial activity of medicaments is particularly important because complete mechanical cleaning of the intricate root canal systems is difficult.

Despite the recognized importance of this, there has been little clinical attention given to the possibility of antimicrobial activity loss from pharmacological interactions when one medicament is used in combination with another. Such commonly used dyadic combinations include antibiotic-corticosteroid pastes and calcium hydroxide (Heithersay 1977; 1984; 1985; Abbott et al. 1989), antibiotic-corticosteroid paste and iodoform (Craig et al. 1987), and calcium hydroxide with iodoform (Fuchino 1980; Matsuzaki et al. 1987).

Although this study employed only two strains of bacteria, they are representative of those commonly isolated from infected primary teeth (Marsh and Largent 1967; Tomic´-Karovic´ and Jelinek 1971; MacFarlane and Samaranayake 1989;). Hence, although it is possible that other oral bacteria not tested may show different sensitivities to the medicaments, the majority are likely to be similarly affected. The results of the present study indicate that the antimicrobial activity of some medicaments may be severely affected by the addition of another compound. This was observed

clearly in the dyadic combinations of Ledermix and Calyxl where the addition of only 25% by volume of Calyxl, to Ledermix converted the zone of complete growth inhibition originally seen in Ledermix to one of only partial inhibition.

These effects are likely the result of pharmacological inactivation of the antibiotic (tetracycline) in Ledermix by Calyxl components.

Thus, our results indicate that dyadic combinations of Ledermix and Calyxl should be avoided for clinical use if the full antimicrobial potential of Ledermix is required. It is also possible that loss of activity of the steroid component occurs; this was not tested in the present investigation, however.

Similar results were observed in the experiments involving dyadic combinations of Kri and Calyxl (Table 5). There is significant loss of antimicrobial activity of Kri when Calyxl was added, indicating that these two medicaments should not be mixed.

In addition, the present study also shows that when two endodontic medicaments with strong antimicrobial activity are combined, there is usually no additive or synergistic effect.

When Ledermix was combined with Kri, microbial growth inhibition at all concentrations of Kri and Ledermix was similar to that observed at 100% Kri alone or with 100% Ledermix alone. These results indicate there is no clinical advantage in adding Kri to Ledermix to increase the antimicrobial activity.

The experiments involving eugenol and formocresol revealed that eugenol showed considerably less antimicrobial activity compared with formocresol (Table 6). However the addition of 25% by volume of formocresol into eugenol increases microbial growth inhibition to that achieved with full-strength formocresol. The clinical implication of this observation is that in the pulpotomy procedure, the standard final pulp dressing of 50% formocresol and 50% eugenol may be replaced by one of 25% formocresol and 75% eugenol with no decrease in antimicrobial activity and less toxicity from formocresol.

In conclusion, the present investigation has demonstrated clearly that many endodontic medicaments should not be used in combination, since the antimicrobial activities of individual components may be affected seriously. Rather, it is recommended that each medicament be used separately to ensure that its individual beneficial potential is not compromised. For example, in an infected immature incisor, an antibiotic paste may be used as a first dressing to control initial presenting infection, followed by calcium hydroxide to induce apexification when the infection is controlled fully. Attempting to achieve two clinical ideals simultaneously with mixtures of medicaments may result only in limited success.

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Patients don't want to be treated by a doctor with AIDS

Most Americans said they would look for a new doctor if they found out their family physician was infected with the AIDS virus, according to a recent survey.

A team of researchers at the University of California at San Francisco interviewed 2000 Americans nationwide, seeking opinions about HIV infection, the cause of AIDS. Fifty-six per cent of those called said they would change physicians if they learned their doctor had HIV infection, and 25% said they'd switch from a doctor they believed was treating patients with HIV infection.

The latter finding is especially disturbing, because the need is growing for physicians from all specialties to provide care to AIDS patients. Some doctors are reluctant to treat HIV patients for fear it will hurt their regular practice. If healthy people shun doctors who merely treat HIV-infected patients, it could add to the growing burden of providing medical care for AIDS patients, the researchers concluded.

Interestingly, many people who said they would leave the practice of a physician infected with HIV also said they know the virus wouldn't be transmitted through contact in the doctor's office.

Even though knowledge about AIDS is growing and most people understand that the infection doesn't spread through casual contact, survey results demonstrate the need for more public education about AIDS.