Microbiological effectiveness of a reduced concentration of Buckley's formocresol

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Abstract

The use of formocresol for the treatment of pulps of primary teeth has evolved empirically. An in vitro study was carried out to test the effectiveness of reduced concentrations of the drug. It was found that the solution of Buckley's formocresol was bacteriostatic at a concentration of between 0.025 and 0.020% formocresol and was bactericidal at a concentration of between 0.50 and 0.33% formocresol on cultures of Streptococcus faecalis, Streptococcus salivarius, and Staphylococcus aureus. S. faecalis was found to be the most resistant organism of those tested after 72 hours. Further investigations in vivo are required to assess the clinical effectiveness of reduced concentrations of formocresol in vital and nonvital primary teeth.

L he use of formaldehyde for the disinfection of inflamed pulps first was reported by Lepkowski in 1897.¹ This technique caused intolerable pain, but it was not until 1904 that a modified formula was introduced by Buckley.² This latter material contained tricresol and glycerine on an empiric basis (rather than on a chemical or biological basis) and was clinically more acceptable.

The endodontic treatment of primary teeth has evolved basically upon empirical methods and is still an unresolved biological problem. Nonetheless, the dominating pulp treatment of vital and nonvital primary teeth is the pulpotomy,³ and clinical success has been reported.⁴⁻⁶

Concern has been expressed about the toxicity, concentration, and systemic distribution of formocresol following pulpotomy and pulpectomy procedures.^{7,8} Lewis and Chestner⁹ have reported on the mutagenic and carcinogenic potential of formalde-hyde on drosophila, grasshoppers, and fungi, but the

findings are not conclusive for mammals. Formocresol even may act locally, leading to the developmental arrest of the succedaneous tooth.¹⁰⁻¹²

Recently, Garcia-Godoy et al.¹³ used a 1 min application of full strength formocresol, and found that it produced less inflammatory response and tissue reaction when compared with 3 min and 5 min applications.

The cytotoxic effects of formocresol, although known to earlier workers, were not fully understood and were based upon subjective criteria. Buckley² stated that there was no necessity to use formaldehyde in the same strength in nonvital teeth containing nonsuppurative material as in teeth which contained putrescent material. Straffon and Han¹⁴ concluded that a lesser concentration of 1/50 formocresol solution did not interfere with the prolonged recovery of connective tissue and that it might have suppressed the initial inflammatory response. Loos and Han¹⁵ concluded that a 1/5 concentration of Buckley's formocresol was as effective as the full strength formula, and allowed for a faster recovery of the affected cells and therefore represented a safer medicament. Gazi et al.¹⁶ found that a 50% dilution of formocresol in propylene glycol was significantly less irritating than a full-strength formula.

The present study was designed to assess the efficacy of reduced concentrations of Buckley's formocresol with time on 3 microorganisms, each of which has been reported to be present in the pulps of infected primary molars. The organisms studied were local isolates of *Streptococcus faecalis*, *Staphylococcus aureus* found in the infected primary molars (used by Wesley et al.¹⁷ and others¹⁸⁻²⁰), and *Streptococcus salivarius* which was found in 70% of infected primary molars by Cohen et al.²¹ and 10-30% by Brook et al.²²

Methods and Materials

The effect of reduced concentrations of Buckley's formocresol^a was investigated using serial dilution in Brain Heart Infusion agar (BHI; BBL)

The experiment was designed to study the bactericidal and bacteriostatic nature of formocresol. The microorganisms in the study were subcultured and plated at regular intervals to ensure purity.

Initially, a broad screening method assessed the growth of *S. faecalis*, *S. salivarius*, and *Staph. aureus* in various concentrations of Buckley's formocresol with BHI agar. The study was carried out in 3 phases.

First, a broad screening phase involved a control BHI agar plate and 5 concentrations (10.00, 1.00, 0.20, 0.01, and 0.001%) of Buckley's formocresol in BHI agar. The Petri dishes were divided into thirds and an overnight broth culture of each microorganism was plated onto each third. The plates were incubated

TABLE 1. Effect of Concentrations of Formocresol on S. faecalis

Concentration of	Growth on Day								
formocresol %	1	2	3	4	5	6			
0.000	+ +	+ +	+ +						
0.100	+ +	+ +	+ +						
0.125	-	+ +	+ +						
0.134		+ +	+ +						
0.167		+	+ +						
0.200			+						
0.250	~	_		(++)	(++)	(++)			
0.333			—	(++)	(++)	(++)			
0.500		—	_	(-)	(—)	(-)			
1.00		-	-	(-)	(-)	(-)			

Organisms were cultured for 3 days and observed directly (visual). + + indicates growth > 10% of the field; + indicates growth < 10% of the field; and - indicates no growth.

 TABLE 2. Effect of Concentrations of Formocresol on S. salivarius

Concentration of formocresol %	Growth on Day							
	1	2	3	4	5	6		
0.000	+ +	+ +	+ +					
0.100	+ +	+ +	+ +					
0.125	~	+ +	+ +					
0.134	-	+	+ +					
0.167	- ۱	-	+					
0.200	-	-	-	(++)	(++)	(++)		
0.250	-		-	(+)	(+)	(++)		
0.333		-		(-)	(—)	(-)		
0.500	-	-		(-)	(–)	(–)		
1.00	-	-	-	(—)	(-)	(–)		

Organisms were cultured for 3 days and observed directly (visual): + + indicates growth > 10% of the field; + indicates growth < 10% of the field; and - indicates no growth.

^a Buckley's formocresol: formaldehyde 19 ml 37%; cresol b.p. 35 ml; glycerine 46 ml.

 TABLE 3. Effect of Concentrations of Formocresol on Staph.

 aureus

Concentration of formocresol %	Growth on Day						
	1	2	3	4	5	6	
0.000	+ +	+ +	+ +				
0.100	+ +	+ +	+ +				
0.125		+ +	+ +				
0.134		+ +	+ +				
0.167	—	+	+ +				
0.200	-	-	+				
0.250	-	-	-	(+)	(+)	(+)	
0.333	-	-	-	(–)	()	(-)	
0.500	_	-	-	(-)	()	(-)	
1.00	-	-	-	(—)	(~)	(-)	

Organisms were cultured for 3 days and observed directly (visual): + + indicates growth > 10% of the field; + indicates growth < 10% of the field; and - indicates no growth.

aerobically at 37° C and were observed directly at 24hr intervals for 72 hr. Microscopic analysis of the agar plates for colony growth was not done. The microorganisms failed to grow at a concentration of 0.2% Buckley's formocresol after 48 hr, but there was growth of *Staph. aureus* and *S. faecalis* after 72 hr at the same concentration.

A similar procedure was carried out in a narrow screen using a control BHI agar plate and 9 concentrations of Buckley's formocresol (1.0, 0.50, 0.33, 0.25, 0.20, 0.167, 0.134, 0.125, and 0.01%). The plates were incubated aerobically at 37° C and observed at 24-hr intervals for 3 days.

After 72 hr the microorganisms on plates which showed no growth were subcultured with a sterile swab onto plates of BHI agar without formocresol to differentiate between the bacteriostatic and bactericidal actions of the drug. The subcultures of organisms from the plates of 1.00, 0.05, 0.33, and 0.25% formocresol in BHI agar were incubated at 37° C aerobically and observed at 24-hr intervals for 72 hr.

The investigation was limited to short periods of 3 days to avoid the possibility of contamination which may have provided false readings if the subculturing had been done after a period of 7 days.

Results

S. faecalis was the most resistant of the 3 microorganisms tested and under the above conditions Buckley's formocresol was found to be bactericidal to a minimum concentration of between .05 and 0.33% formocresol in BHI agar (Table 1).

S. salivarius showed a similar pattern to *S. faecalis*, but growth in a concentration of 0.333% Buckley's formocresol was less marked and the growth of both organisms in a concentration of 0.250% Buckley's formocresol was similar (Table 2).

Table 3 shows the effect of varying concentrations of formocresol on *Staph. aureus* which shows no growth at a concentration of 0.333% Buckley's formocresol and marginal growth at a concentration of 0.250% formocresol.

Discussion

In the past, many tissue toxic medications were used in an effort to destroy all microorganisms.²³ Schilder²⁴ stated that while drugs killed microorganisms they also may destroy cells and tissue, and that drugs should be selected on the basis of tissue tolerance rather than on antimicrobial activity. A number of investigators have observed the necessity for a reduced concentration of formocresol which would avoid the delay in recovery of normal biological activities of affected connective tissue cells.^{14,17,25}

The present findings indicated that Buckley's formocresol was bacteriostatic to a minimum concentration of between 0.25 and 0.20% and bactericidal to a minimum concentration of between 0.50 and 0.33%.

Apparently only the bacteriostatic potential of Buckley's formocresol in the liquid or vapor form has been documented. It appears that authors¹⁷ who produced areas of no growth or "sterility" due to the bacteriostatic nature of formocresol may not have realized that they produced inhibition of growth, which, if subcultured onto BHI agar without formocresol, would have demonstrated growth. It should be pointed out that in the present investigation a selective inhibitor was not used on plates on which regrowth was carried out, so contaminants could not be identified positively. However, the experiment was repeated 3 times and the results were consistent.

The present investigation may suggest that a different approach to formocresol sensitivity and tissue tolerance is needed. The results confirm the bacteriostatic study of Wesley et al.¹⁷ who found that when relying on vapor alone, a minimal effective dose of 0.004 ml of formocresol was required to inhibit *S. faecalis* after 48 hr, while *Staph. aureus* was similarly inhibited with a much smaller dose of 0.0025 ml under similar conditions.

S. faecalis was the most resistant organism of those tested and has been found to be the most difficult organism to eradicate from root canals.²⁶⁻²⁸

At a concentration of between 0.05 and 0.33% formocresol the drug was an effective bactericidal agent after 72 hr, which confirms the work of Treanor and Goldman.²⁹ However, they also concluded that formocresol failed to completely sterilize the root canal. Hence, it may be possible to mechanically debride the pulp chamber during a pulpotomy procedure, apply a bacteriostatic concentration of the drug, and rely on the host's defense mechanisms to overcome any infection.

Also, it may be possible that infected primary teeth treated by a 2-stage procedure may require the second stage to be completed after 72 hr instead of the empirically based 7 days which has been advocated. One could speculate that this shorter procedure may avoid the possibility of reinfection or secondary infection which may require retreatment.

A long-term study in vivo is the next logical step for the investigation of the success and failure rates, histological confirmation, and a better understanding of the pharmacology of formocresol.

Conclusion

The microbiological effectiveness of varying reduced concentrations of Buckley's formocresol was investigated in vitro on cultures of *S. faecalis, S. salivarius* and *Staph. aureus*. Reduced concentrations of between 0.05 and 0.33% formocresol (dilution 1/200 and 1/300) were found to be bactericidal on those microorganisms tested. *S. faecalis* was found to be the most resistant organism of those tested after 72 hr. Further bacteriological investigations in vivo are required to assess the clinical effectiveness of reduced concentrations of Buckley's formocresol on both vital and nonvital primary teeth.

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Quotable quote: the first cause

...psychologists Sandra Scarr of the University of Virginia and Kathleen McCartney of Harvard University are championing a new and provocative theory of child development.

The theory, its authors explain, presupposes that the stages of our psychological development are set genetically and acted upon environmentally. Scarr and McCartney argue that each and every stage of a child's psychological development is ushered in by an increment in the child's biological maturation — that there is no evidence for its ever being induced by environment alone. Only after a child is genetically receptive, the theory maintains, is the environment able to have any real effect on his or her behavioral development. Guillen MA: The first cause. Psych Today

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