PEDIATRIC DENTISTRY/Copyright ©1985 by
The American Academy of Pediatric Dentistry
Volume 7 Number 3

The diffusion of glutaraldehyde from zinc oxide-eugenol cement

Don M. Ranly, DDS, PhD Franklin Garcia-Godoy, DDS, MS

Abstract

The diffusion of glutaraldehyde from zinc oxide and eugenol cement (ZOE) into water was measured. The egress was found to be substantial and correlated to the amount of glutaraldehyde which was incorporated initially. The percentages of the fixative which diffused from the ZOE preparations containing 2,5, or 10% glutaraldehyde were virtually identical, demonstrating that there is no restriction of movement imposed by the matrix of the cement. The results suggest that ZOE might be used as a vehicle to introduce glutaraldehyde to the radicular pulp of a pulpotomized primary tooth.

The deficiencies of formocresol as a pulpotomy agent in primary teeth have been documented amply in recent years,[1-6] and as a result alternative chemicals have been proposed.[7,8] One of these, glutaraldehyde, a standard fixative used in electron microscopy, has been evaluated in laboratory and clinical investigations.[9-11] In vitro analyses have demonstrated that glutaraldehyde is an excellent fixative,[8,12] and the trials in animals[13] and humans[10,11] have been promising. The traditional method of applying formocresol to the amputation site has been a moistened cotton pellet.[14] In some situations it also is incorporated in the zinc oxide and eugenol cement (ZOE) which is used as a base over the fixed radicular tissue.[14] In human teeth, diffusion of formocresol from the cement alone effected pulp changes comparable to those observed following treatment with a formocresol-moistened pellet.[15] A histologic study of monkey teeth treated with a ZOE dressing containing glutaraldehyde suggested that pulp might be fixed adequately by this protocol.[16] Glutaraldehyde was not incorporated into ZOE as an adjunct to treatment in either of the clinical evaluations of glutaraldehyde.[10,11] We believe that ZOE with incorporated glutaraldehyde might serve as an excellent vehicle to introduce the fixative to the tissue, even to the extent that pretreatment with the cotton pellet might be obviated. This study was initiated to determine whether glutaraldehyde has the same diffusibility from ZOE as has been reported for formaldehyde[17] and cresol[18] and whether, therefore, the delivery of glutaraldehyde by the base has potential as a treatment modality.

Methods

Two, 5, and 10% solutions of the fixative were prepared by diluting laboratory-grade glutaraldehyde (25%) with glycerol and water. Glycerol, which was added to facilitate later mixing with the ZOE, constituted 60% by volume of the test solutions.

Zinc oxide was mixed with eugenol and the glutaraldehyde solutions in the ratio: 4 g:1.0 ml:0.5 ml. This combination provided a reasonably stiff mixture for testing. Both zinc oxide and eugenol were laboratory grade.

Plastic vessels (shell vial plastic stoppers) with a diameter of 12 mm and a depth of 10 mm which contained approximately 2.6 g of cement were used to test the diffusion of glutaraldehyde. The vessels were weighed prior to filling. Sufficient cement was mixed to fill all the vessels in a test group. After careful filling to eliminate voids, they were inverted with pressure on glass to ensure a flat and smooth surface. Allowed to set in this inverted position, the vessels then were removed, carefully cleaned to eliminate flash, and reweighed. The difference in weight allowed an accurate determination of the cement content of each vessel, and indirectly, by appropriate calculations, the total quantity of glutaraldehyde. Each

vessel then was submerged in a sealed bottle containing 12 ml of distilled water. Each test group was composed of 5 samples.

At 1,3,7,14, and 25 days, duplicate 200 µl samples were taken from the vials and assayed using Schiff's reagent. Standards were prepared from the same glutaraldehyde that was used in the preparation of the test solutions. Although the standard curve for the colored products formed by glutaraldehyde and Schiff's reagent was demonstrated to be definite and reproducible, a straight line was not obtained either when the transmission was plotted against the concentration or when the logarithm of the transmission was so plotted. This phenomenon is apparently characteristic of the reaction of aldehydes with fuchsin-sulfite, having been reported many years ago for formaldehyde.[19] From the glutaraldehyde content of the assayed samples, the total quantity which diffused into each storage vessel was calculated. Using these values and the weights of the material in the vessels, the diffusion of glutaraldehyde into the water is presented as µg of glutaraldehyde/g of cement. The amount of glutaraldehyde lost in the assay procedures was recorded and included in the accumulated values for the appropriate time periods.

Results

Table 1 presents the values for glutaraldehyde which diffused from 1 g of ZOE cement under the experimental conditions. Table 2 expresses the diffusion as a percentage of the total glutaldehyde incorporated in the vessels. It is apparent that the quantity of the fixative that was released into the water correlates to its original concentration in the cement. When the values are graphed (Fig 1), the curves follow the same pattern, but differ significantly in absolute quantities. The curves demonstrate that the movement of glutaraldehyde into the water media is at first rapid, but that the rate of diffusion progressively slows, and sometime after the third day the egress reaches a steady state.

Table 1. The Diffusion of Glutaraldehyde From ZOE Cement Expressed as µg of Glutaraldehyde/g of Cement. Values Represent the Mean ± SEM.

	µg/g Glutaraldehyde Released				
Day	*1*	*3*	*7*	*14*	*25*
2%	360 ±34	496 ±23	746 ±65	974 ±84	1311 ±92
5%	794 ±98	1274 ±207	1660 ±80	2231 ±143	3198 ±173
10%	1250 ±88	2146 ±306	2968 ±203	4083 ±364	6077 ±515

Table 2. The Diffusion of Glutaraldehyde From ZOE Cement Expressed as a Percentage of the Total Incorporated. Values Represent the Mean ± SEM.

	% Glutaraldehyde Released				
Day	*1*	*3*	*7*	*14*	*25*
2%	18 ±1.7	25 ±1.2	37 ±3.1	49 ±4.0	66 ±4.2
5%	18 ±2.1	28 ±4.5	37 ±2.1	50 ±3.14	71 ±3.8
10%	14 ±1.0	24 ±3.4	33 ±2.7	45 ±4.3	67 ±5.5

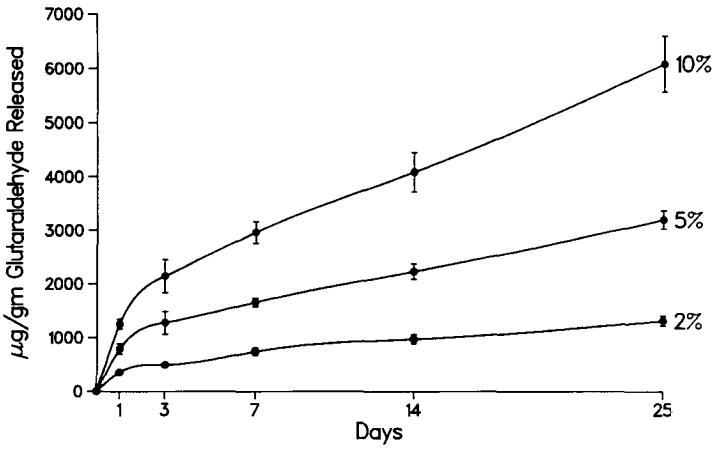

Fig 1. The diffusion of glutaraldehyde from ZOE expressed as µg glutaraldehyde/g of ZOE cement.

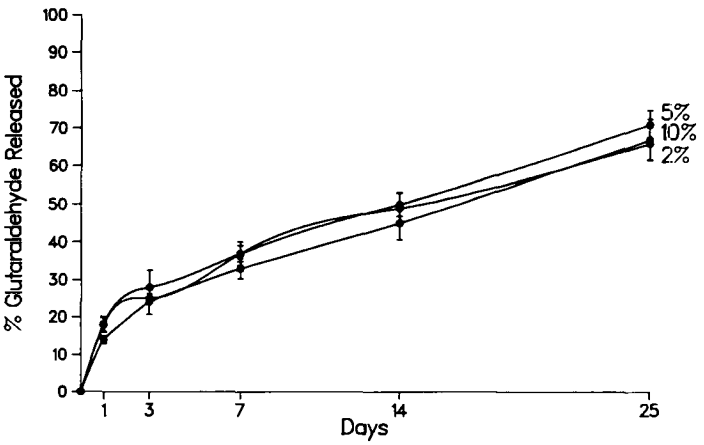

Fig 2. The diffusion of glutaraldehyde from ZOE expressed as a per cent of the total incorporated.

In Figure 2 the release of glutaraldehyde is depicted as a percentage of the original quantity incorporated in the vessels. At the termination of the study at day 25, more than 65% of the incorporated glutaraldehyde in each test group had diffused into the water.

Discussion

The results of this study clearly demonstrate that glutaraldehyde neither reacts chemically with the constituents of ZOE or is bound physically by its matrix. The quantity which diffused from the cement was substantial and did not reach an end point in the time frame of this experiment. Furthermore, the amount of the fixative which escaped correlated well with the original concentration in ZOE mix.

Judging from the virtual overlap of the percentage curves, the release of glutaraldehyde clearly is not dose dependent; a constant proportion of the fixative diffused from all the vessels, regardless of the starting concentrations.

This study did not include formocresol, since its diffusion characteristics were investigated previously.[17,18] These earlier studies demonstrated that virtually all of the formaldehyde that diffused from ZOE did so within 1 week. Glutaraldehyde, as demonstrated in this study, was considerably slower to egress. This difference might be explained by the larger molecular size of glutaraldehyde and a simple physical hindrance.

The rate and extent of diffusion of glutaraldehyde in an in vivo situation cannot be extrapolated from the values obtained in this experiment. Obviously, the aqueous environment was maximized in the study protocol, a condition which would not be expected in the pulp. Although tissue fluid could be anticipated to provide a medium for glutaraldehyde diffusion, it might be limited by the disease state, or even restrictively sealed off by the cross-linking properties of glutaraldehyde itself. A localized distribution of the fixative into the radicular pulp might be ideal; on the other hand, a superficial reaction with the tissue might not be sufficient to ensure asepsis, detoxification, inhibition of autolytic enzymes, and suppression of resorptive activity. The efficacy of the ZOE delivery system can be completely tested only in vivo. However, this study demonstrates that any limitations which might develop in a clinical trial could not be ascribed to the failure of ZOE to release glutaraldehyde.

This research was funded by NIDR grant DE06446.

The authors acknowledge the expert technical assistance of Diane Horn.

Dr. Ranly is a professor and Dr. Garcia-Godoy is associate professor, pediatric dentistry, Dental School, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284. Reprint requests should be sent to Dr. Ranly.

1. Loos PJ, Straffon LH, Han SS: Biologic effects of formocresol. J Dent Child 40:193–97, 1973.
2. Block RM, Lewis RD, Sheats JB, Burke SG: Antibody formation to dog pulp tissue altered by formocresol within the root canal. Oral Surg 45:282–92, 1978.
3. Rolling I, Lambjerg-Hansen H: Pulp condition of successfully formocresol-treated primary molars. Scand J Dent Res 86:267–72, 1978.
4. Pruhs RJ, Olen GA, Sharma PS: Relationship between formocresol pulpotomies of primary teeth and enamel defects on their permanent successors. JADA 94:698–700, 1977.
5. Myers DR, Pashley DH, Whitford GM, McKinney RV: Tissue changes induced by the absorption of formocresol from pulpotomy sites in dogs. Pediatr Dent 5:6–8, 1983.
6. Lewis BB, Chestner SB: Formaldehyde in dentistry: a review of mutagenic and carcinogenic potential. JADA 103:429–34,1983.
7. s'-Gravenmade EJ: Some biochemical considerations of fixation in endodontics. J Endod 1:233–37, 1975.
8. Ranly DM, Lazzari EP: A biochemical study of two bifunctional reagents as alternatives to formocresol. J Dent Res 62:1054–57, 1983.
9. Nelson JR, Lazzari EP, Ranly DM, Madden RM: Biochemical effects of tissue fixatives on bovine pulp. J Endod 5:139–44, 1979.
10. Kopel HM, Bernick S, Zachrisson E, De Romero SA: The effects of glutaraldehyde on primary pulp tissue following coronal amputation: an in vivo histologic study. J Dent Child 47:425–30, 1980.
11. Garcia-Godoy F: Clinical evaluation of glutaraldehyde pulpotomies in primary teeth. Acta Odontol Pediatr 4:41–44, 1983.
12. Ranly DM: A comparative study of the effects of formaldehyde, glutaraldehyde, and dimethylsuberimidate on enzyme activity in the bovine dental pulp. Acta Odontol Pediatr 5:5–8, 1984.
13. Davis MJ, Myers DR, Switkes MD: Glutaraldehyde: an alternative to formocresol for vital pulp therapy. J Dent Child 49:176–80, 1982.
14. Berson RB, Good DL: Pulp therapy. In Pediatric Dentistry, Stewart RE, ed. St Louis; CV Mosby Co, 1982 Ch 62.
15. Mejare I, Hasselgren G, Hammarstrom LE: Effect of formaldehyde-containing drugs on human dental pulp evaluated by an enzyme histochemical technique. Scand J Dent Res 84:29–36, 1976.
16. Tagger E, Tagger M: Pulpal and periapical reactions to glutaraldehyde and paraformaldehyde pulpotomy dressing in monkeys. J Endod 10:364–71, 1984.
17. Ranly DM, Pope HO: An in vitro comparison of the release of ^{3}H-formaldehyde and ^{14}C-P-Creosol from zinc oxide-eugenol cement. Pharmacol Ther Dent 4:53–57, 1979.
18. Hoffpauir CL, Buckaloo GW, Guthrile JD: Determination of combined formaldehyde in organic compound and in cellulose formals. Ind Eng Chem, Anal Ed 15:605–6, 1943.