

The effects of various concentrations and lengths of application of glutaraldehyde on monkey pulp tissue

J. Michael Lloyd, DDS, MSD N. Sue Seale, DDS, MSD
Carolyn F.G. Wilson, DDS, MSD

Abstract

This study was designed to examine the histologic response of the dental pulp to the use of selected concentrations of glutaraldehyde applied over various time intervals in pulpotomies performed on monkey teeth. One hundred sixty primary teeth from 8 stump-tail macaque monkeys were divided randomly into 10 groups. One group served as the control and received a zinc oxide and eugenol pulpotomy with no glutaraldehyde. The other 9 groups represented pulpotomies treated with 0.5, 1.0, or 2.0% glutaraldehyde applied for 2, 5, or 10 min. The teeth were assigned to observation groups of 1 day, or 1, 4, or 8 weeks. The monkeys were perfused following termination of the study and the maxillae and mandibulae were removed en bloc. The teeth were examined for histologic changes in the tissues associated with each concentration and time of application. One day after glutaraldehyde treatment, all samples had a zone of fixed pulp tissue at the application interface. At 1 week and continuing through 8 weeks, findings included moderate chronic inflammation progressing to severe inflammation with eventual giant cell formation and internal resorption. The severity of the reactions was related to medicament concentration and time of application with the lower concentrations and shorter application time revealing more severe inflammatory responses.

In recent years, formocresol has diminished in popularity as the primary medicament used in vital pulpotomies because of the documented problems of systemic absorption and concurrent systemic toxic effects,¹ locally toxic effects,² and less than ideal clinical and histological effectiveness.³ There is a need to evaluate alternative agents which are both clinically and biologically more successful, and glutaraldehyde has been suggested as an alternative to formocresol in pulpotomy

procedures.⁴ It is a chemically bifunctional reagent which is a common fixative in electron microscopy (Dawes 1979). Penetration into surrounding periapical tissues is limited primarily by glutaraldehyde's protein cross-linkage formation, and thus, systemic distribution of glutaraldehyde is limited (Davis et al. 1982). Clinical and/or histologic success has been shown to be comparable if not superior to formocresol in various pulpotomy and endodontic procedures.⁵

Although glutaraldehyde was previously used successfully in pulpotomy procedures, there was considerable variability in the application techniques, and they all appear to be empirically determined. Reports of 2-5% glutaraldehyde solutions were common with application times ranging from 1 to 5 min.⁶ The purpose of this study was to determine whether the histologic response of pulp tissue would vary significantly according to the concentration of the glutaraldehyde and length of time it was applied.

Materials and Methods

The study sample consisted of all 20 erupted primary teeth from each of eight 12 to 18-month-old, stump-tailed macaque monkeys. Utilizing a random sampling technique, the 160 teeth were equally distributed into 10 groups (9 experimental, 1 control) which represented the treatment modalities (Table 1 — next page). There were 4 treatment intervals for the pulpotomy procedures prior to animal sacrifice (8 weeks, 4 weeks, 1 week, and 1 day) resulting in 40 groups of 4 teeth each.

The animals were anesthetized using ketamine hydrochloride at approximately 20 mg/kg. One-half of the initial dose of ketamine hydrochloride was repeated every 30-40 min to maintain adequate anesthesia.

¹ Myers et al. 1978; Pashley et al. 1980; Myers et al. 1981; Myers et al. 1983.

² Pruhs et al. 1977; Block et al. 1977; Block et al. 1978.

³ Rolling et al. 1976; Magnusson 1978; Rolling and Lambjorg-Hansen 1978.

⁴ Davis et al. 1982; Garcia-Godoy 1983; Lekka et al. 1984; Tagger 1984; Fuks et al. 1986; Garcia-Godoy 1986.

⁵ Davis et al. 1982; Wemes et al. 1982; Garcia-Godoy 1986; Tagger 1984; Fuks et al. 1986.

⁶ Davis et al. 1982; Tagger 1984; Garcia-Godoy 1986; Fuks et al. 1986; Myers et al. 1986.

Preoperative radiographs showed all teeth to be fully rooted with apices closed and free of lesions. A clinical examination prior to operative procedures indicated that all teeth were intact and free of carious lesions.

A rubber dam was used to isolate teeth, and a modified Class I cavity preparation was made over the pulp chamber using a high-speed, no. 330 carbide bur. The roof over the chamber was removed with the high-speed bur, and the coronal pulp tissue was removed using a slow speed no. 4 round bur and a sterile spoon excavator. The pulp chamber was cleansed and hemostasis obtained using water and sterile cotton pellets. The teeth were treated as follows.

Experimental Teeth

The pulp chambers were filled with one drop of either 0.5, 1.0, or 2.0% glutaraldehyde solution which remained in place for 2, 5, or 10 min. The solutions were brought to a pH of 7.3 with a phosphate buffer (0.146 M) to approach normal body pH. The solutions were removed by blotting with sterile cotton pellets. A paste consisting of reinforced zinc oxide powder, one drop of the respective concentration of glutaraldehyde, and one drop of eugenol was placed over the pulp stumps and gently pressed into place using a sterile amalgam condenser. An amalgam restoration was placed over the base material.

Control Teeth

A paste consisting of reinforced zinc oxide powder and one drop of eugenol was placed over the pulp stumps and gently pressed into place using a sterile amalgam condenser. A final restoration of amalgam was condensed into the preparation once the original base had set.

One day following the final treatment interval, all 8 animals were anesthetized and perfused first with saline and then a phosphate buffered glutaraldehyde (GTA-PBF) solution according to the technique described by Cox et al. (1977). The mandibulae and maxillae were removed en bloc. Each bone was cut vertically to produce 2 blocks of anterior and 2 blocks of posterior teeth. The blocks were placed in separate bottles of GTA-PBF for fixation for 2 weeks and demineralized in EDTA, pH 7.15, for 2 months and monitored radiographically. The tissue blocks were dehydrated in graded alcohol, embedded in paraffin, and serially sectioned at 5 μ thickness. Every tenth slide was collected, stained with H & E, and examined histologically by 2 investigators for staining properties, presence and type of inflammatory response, reparative dentin formation, and internal resorption. The investigators did not know from which treatment groups the sections were taken. Radiographs were made of each block section prior to

TABLE 1. Distribution of Teeth Among Treatment Groups (N = 160)

GRP/Treatment*	Observation Time				Total
	1 Day	1 Week	4 Weeks	8 Weeks	
I 2 min 0.5%	4	4	4	4	16
II 5 min 0.5	4	4	4	4	16
III 10 min 0.5	4	4	4	4	16
IV 2 min 1.0	4	4	4	4	16
V 5 min 1.0	4	4	4	4	16
VI 10 min 1.0	4	4	4	4	16
VII 2 min 2.0	4	4	4	4	16
VIII 5 min 2.0	4	4	4	4	16
IX 10 min 2.0%	4	4	4	4	16
X Control ZOE†	4	4	4	4	16
Total	40	40	40	40	160

* % indicates concentration of glutaraldehyde.

† ZOE indicates zinc-oxide and eugenol control.

demineralization. The radiographs were examined for periapical or bifurcation radiolucencies, and internal or external resorption.

Results

Clinical Results

All 160 teeth observed clinically in this study were considered to be successful as determined by the absence of soft tissue changes, sinus tracts, and mobility.

Radiographic Results

Of the 160 teeth radiographed when the monkeys were sacrificed, 12 showed severe internal resorption (Fig 1).

Histologic Results

One hundred fifty-nine teeth were examined for histologic changes. One tooth was lost due to improper sectioning.



FIG 1. Internal resorption in a mandibular central incisor at 8-week observation period.

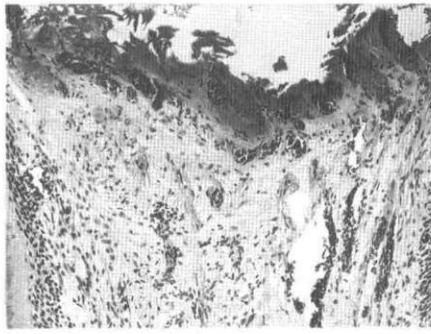


Fig 2. (left) One-day observation period — 0.5% glutaraldehyde, 2 min. Eosinophilic staining zone (darker band in upper one-third of figure) of fixation containing vessels filled with red blood cells. Pulp tissue subjacent to the eosinophilic zone is normal. (180x)

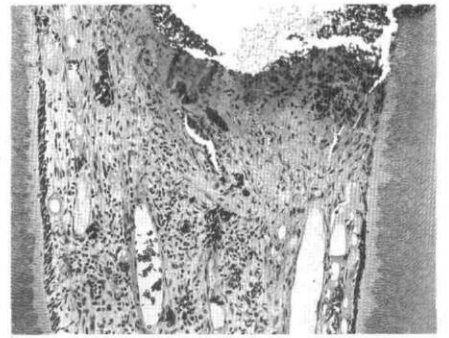


FIG 3. (right) One-week observation period — 0.5% glutaraldehyde, 5 min. Eosinophilic staining zone has moderate chronic inflammatory reaction directly beneath it. (180x)

1-Day Observation Period — 36 Teeth. All teeth in the one-day observation period were similar in appearance with an eosinophilic staining zone of pulp tissue at the medicament interface. This zone was amorphous with scattered cell nuclei. The only variation between treatment groups appeared to be the width of this zone. The zone was very narrow in the 0.5%, 1-min application group (Fig 2) and became wider as the medicament concentration and time of application increased. The widest zone was visible in the 2.0%, 10-min group. The pulp tissue beneath this zone appeared normal and undisturbed in all teeth.

1-Week Observation Period — 36 Teeth. The coronal pulp tissue showed greater evidence of response to the chemical treatment by one week. The response consisted of attempts at removal and replacement of the eosinophilic staining zone and inflammatory infiltrate of the coronal one-third (Fig 3). The intensity of the replacement response appeared related once again to the medicament concentration and length of application. The pulps of the teeth treated with the lower concentrations and lesser application times revealed more active replacement and greater inflammation. One pulp was necrotic in the 2.0%, 2-min application time group. The teeth treated with higher concentrations of glutaraldehyde for longer periods of time had the widest eosinophilic staining zone and were less affected by an inflammatory response (Fig 4). The 2.0%, 10-min application time group revealed the least inflammatory reaction. The inflammatory reaction ap-

peared to be confined to the coronal one-third while the middle and apical thirds were normal in all but 2 of the 36 teeth in this observation time period.

4-Week Observation Period — 36 Teeth. By 4 weeks' observation time, the pulpal response had become more aggressive, and giant cell formation and some beginning internal resorption were evident. Chronic inflammatory cell infiltrate was a consistent finding in the pulp tissues in the coronal one-third of all teeth in all treatment groups (Fig 5). The intensity of the reactions continued to be related to the medicament concentration and length of application with more intense reactions observed in the pulps of teeth treated with lower concentrations and for lesser times of application. The eosinophilic staining zone was still present to a significant degree only in the teeth treated with the higher concentrations for longer application times. The inflammatory reaction had spread to involve the middle third in most teeth, but the apical tissues were normal with the exception of five teeth which demonstrated scattered inflammatory cells. The zinc oxide and eugenol control teeth were heavily infiltrated with inflammatory cells in the coronal one-third and internal resorption was beginning in some teeth.

8-Week Observation Period — 35 Teeth. By 8 weeks, total removal of the eosinophilic staining zone and active, aggressive internal resorption were common findings in the teeth treated with lower concentrations and for lesser application times (Figs 6, 7 — next page). Severe chronic inflammation and abscess formation in



FIG 4. (left) One-week observation period — 2.0% glutaraldehyde, 2 min. Eosinophilic staining zone has mild chronic inflammatory cell infiltrate directly beneath it. (180x)

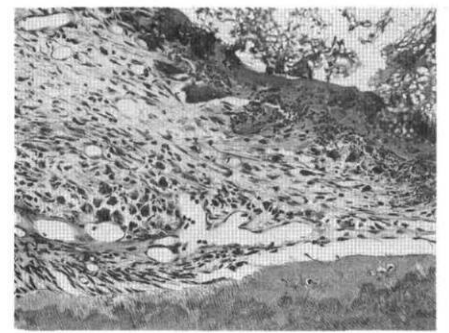


FIG 5. (right) Four-week observation period — 1.0% glutaraldehyde, 2 min. The eosinophilic zone is present but invaded by phagocytic cells and a moderate chronic inflammatory cell infiltrate. Giant cells also are seen beneath the eosinophilic staining zone. (235x)

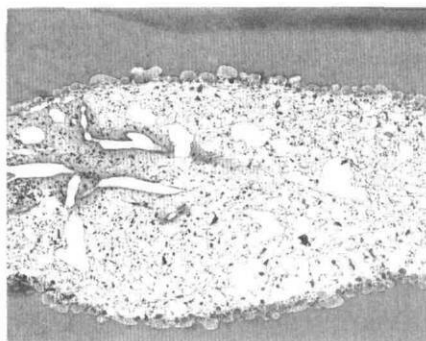
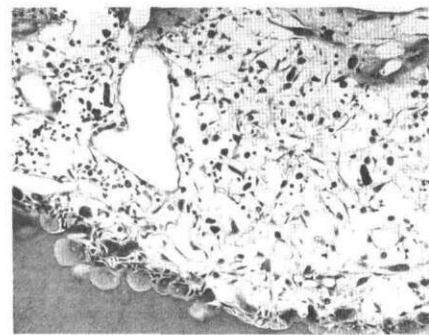


FIG 6. (left) Eight-week observation period—1.0% glutaraldehyde, 2 min. Coronal third of pulp shows no eosinophilic zone present, a severe chronic inflammatory cell reaction, and giant cells undergoing active internal resorption. (72x)

FIG 7. (right) Higher magnification of pulp in Fig. 6 shows inflammatory reaction and active internal resorption. (180x)



the coronal and middle two-thirds also were commonly seen. Two teeth were necrotic in the 5-min application time group, 1 treated with 0.5% and 1 treated with 1.0%. The inflammatory reactions were milder and more confined to the coronal one-third in the teeth treated

with higher concentrations and longer application times of glutaraldehyde. The pulps treated with 2.0% glutaraldehyde for 10 min still contained the eosinophilic staining zone in all 4 teeth (Fig 8). The control teeth containing zinc oxide and eugenol demonstrated varying degrees of degener-

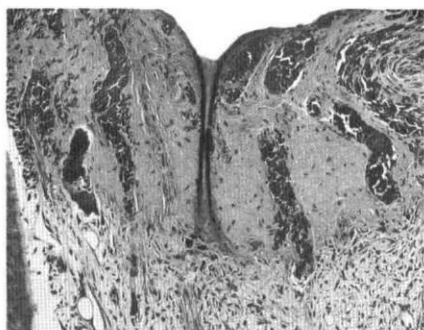


FIG 8. Eight-week observation period—2.0% glutaraldehyde, 10 min. Wide eosinophilic staining zone is still present. (180x)

eration with vacuolization in the odontoblastic layer.

Discussion

The clinical objective of the pulpotomy in primary teeth is to retain the tooth until normal resorption and exfoliation occurs. Clinically, all teeth in this study appeared successful for the times observed.

The histologic objective of the pulpotomy procedure utilizing a medicament of the "fixative" type is to create a chemically altered zone at the medicament interface which would be stable over time and leave the deeper, untreated pulp tissue vital and uninfamed. With these treatment goals, the difference in reaction of the pulp tissues based upon concentration of the applied agent and length of application times seen in this study could definitely affect a successful outcome.

At the 1-day observation period there was evidence of an eosinophilic staining zone of pulp tissue at the medicament interface in all experimental teeth which probably represented fixation of the tissue associated with the glutaraldehyde application. The depth of this fixed zone increased with increased concentrations and increased times of application of the glutaraldehyde.

There was no evidence of graded layers of fixation in any of the teeth. Glutaraldehyde has two functional aldehyde groups in its molecular structure, thereby forming more stable bonds with proteins. It is thought that this strong protein cross linking limits pulpal penetration and prevents diffusion to adjacent tissues. At the 1-day interval the remaining tissue apical to the eosinophilic staining zone remained normal with no inflammation and excellent cellular detail.

The control teeth revealed a blood clot at the amputation site and a mild to moderate inflammatory reaction just apical to it. There was no eosinophilic staining zone present. Therefore, this zone was created by the glutaraldehyde and was not a reaction to the zinc oxide and eugenol.

Past the 1-day observation period, definite changes were observed in the fixed zone and the tissue below it. All teeth in the 1-week observation time group began to show a mild to moderate chronic inflammatory cell infiltrate directly beneath the fixed zone. However, the severity of the response was definitely related to the concentration of the glutaraldehyde and length of time it was left in place during the pulpotomy procedure. The 2.0% glutaraldehyde-treated teeth showed less inflammation than did the 0.5 or 1.0% groups. The remainder of the pulp tissue at this time interval remained normal.

By the 4-week interval the fixed zone was beginning to be removed in about half the teeth in this group. Giant cells were first visible in the teeth in this time period and represented attempts to remove this fixed layer of tissue. Thick, deeply stained fibrous tissue was also observed under the fixed zone, indicative of fibroblastic proliferation and replacement of the fixed zone. The first evidence of internal resorption was in several teeth in this group and indicated that the giant cells were not limiting their activity to the fixed tissue. Here again, the severity of this overall response of replacement and internal resorption was inversely proportional to the concentration of the treatment agents and time of application with the 2.0% glutaraldehyde applied for 10 min showing the least amount of inflammation and progressing to more inflammation as the concentration of glutaraldehyde and time of application decreased. The

remainder of the pulp was normal.

At the 8-week interval the fixed zone was much less obvious or absent. Giant cells were present in all teeth and appeared to be responsible for removal of the fixed zone. Fibroblastic proliferation and replacement was evident by the thicker, more eosinophilic staining fibers that had taken the place of the fixed zone. Here again, the 2.0% glutaraldehyde solution demonstrated a more stable fixed zone as opposed to the 0.5 and 1.0% solutions. The fact that the 2.0% glutaraldehyde-treated teeth retained this fixed zone at the amputation site at all time periods suggests that this concentration left a more stable tissue than did the 0.5 or the 1.0% concentrations. This study suggests that possibly a higher concentration of glutaraldehyde would create even more stable tissues over longer periods of time.

When comparing the lengths of application of glutaraldehyde, the 10-min length of application appeared to have more favorable results than did the 2- or 5-min applications. Overall, the longer a given concentration of glutaraldehyde was left in contact with the pulp tissue the more stable the tissue appeared over time.

Because the penetration of glutaraldehyde is limited due to its rapid bonding to proteins, longer contact with the pulp tissue may be necessary for it to be effective at lower concentrations. Even with longer application times, the apical and middle thirds of the pulp were not affected. It is not known whether the teeth treated with 2.0% glutaraldehyde for 10 min would, with longer time of observation, eventually deteriorate to the condition of the other teeth in this study.

It appears from the results of this study that the concentration of the glutaraldehyde solution used in pulpotomy procedures and the time the solutions are left in place definitely affect the stability of the treated tissues and the response of the deeper tissues to the treated zone. Further evaluation of even higher concentrations for shorter periods of time appear warranted.

Conclusions

1. The reaction of pulpal tissue to glutaraldehyde appeared to be related to the concentration and the time of application.
2. The initial reaction was one of tissue fixation with depth of fixation greater with increased concentration and application time.
3. The stability of the fixed tissue to resist removal and replacement over time by the pulp tissue appeared to be greater with increased concentration and increased times of application.
4. The predominant negative findings or cause of failure at later observation times appeared to be internal resorption.

This research project was conducted in partial fulfillment for a

master's of science in dentistry and was funded in part by Baylor College of Dentistry Research Funds.

Dr. Lloyd is in the private practice of pediatric dentistry in Arlington, Texas; Dr. Seale is a professor and chairman and Dr. Wilson is an assistant professor, pediatric dentistry, Baylor College of Dentistry, Dallas, Texas. Reprint requests should be sent to Dr. N. Sue Seale, Baylor College of Dentistry, Dept. of Pediatric Dentistry, 3302 Gaston Ave., Dallas, TX 75246.

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Lasers to replace drills?

In the future, lasers have the potential to replace drills and scalpels in dental offices according to some researchers.

Doctors at Northwestern University Dental School first used the laser in 1983 to vaporize gum tissue that had covered the teeth in patients taking the anticonvulsant drug Dilantin.[®] One side effect of taking this drug is excessive growth of gum tissue.

Lasers reduce bleeding during surgery because they seal blood vessels as they cut. Patients who undergo laser surgery also experience less postoperative pain because the instrument apparently seals nerves. There is also less postoperative infection because the laser sterilizes the mouth.

Doctors also are using lasers for severing the skin attaching the lip to the gum in preparation for braces, vaporizing benign tumors, stripping away tissue overgrowth caused by dentures, obliterating tissue discoloration caused by smoking, cutting out tissue for grafts and biopsies, and even for removing oral cancers.

One endodontist is using a laser instead of a drill to cut off the end of roots that had not responded to conventional root canal therapy.