PEDIATRIC DENTISTRY/Copyright ©1986 by
The American Academy of Pediatric Dentistry
Volume 8 Number 2

Systemic absorption of ^{14}C-glutaraldehyde from glutaraldehyde-treated pulpotomy sites

David R. Myers, DDS, MS David H. Pashley, DDS, PhD
Francis T. Lake, DDS, PhD David Burnham
Sasi Kalathoor, Richard Waters

Abstract

The purpose of this research was to measure the systemic absorption and distribution of glutaraldehyde from pulpotomy sites in dogs.

Pulpotomies were performed on the incisors and canines of 5 mongrel dogs. A cotton pellet containing 20 μl of 2.5% ^{14}C-(1,5) glutaraldehyde with an activity of 6.25×10^5 dpm/μl was placed in each pulpotomy site for 5 min. Whole blood samples, urine, and expired air were collected up to 90 min when the animals were sacrificed and tissue samples removed from various organs. The tissue samples were prepared and counted in a scintillation counter to determine ^{14}C-activity.

The results demonstrate that glutaraldehyde is absorbed into the systemic circulation following 5-min application of the agent to vital pulpotomy sites. Tissue binding of absorbed glutaraldehyde was relatively low and the remaining portion of the absorbed glutaraldehyde was metabolized and excreted in the urine or exhaled as carbon dioxide. Gradual impairment of the microcirculation of the pulp occurred following glutaraldehyde application.

The need for an alternative to formocresol as a pulpotomy agent in primary teeth arises from concern about formocresol's clinical effectiveness,[1-3] local effects,[4-6] systemic absorption,[7,8] and systemic toxicity.[9,10] Glutaraldehyde has been suggested as a potential pulpotomy agent.[11,12] Glutaraldehyde is a bifunctional reagent and is a standard fixative for electron microscopy.[13] It is an effective protein cross-linking agent and therefore a powerful tissue fixative.[14-17] In vivo[18] and in vitro studies[19] report glutaraldehyde diffuses through tooth structure less readily than does formocresol. Glutaraldehyde also has been shown to diffuse readily from zinc oxide-eugenol cement.[20] Animal studies suggest glutaraldehyde is more active in fixing surface pulp tissue and diffuses less readily through the pulp than does formocresol.[12] Although limited investigations report favorable clinical results,[21,22] so little information is available concerning glutaraldehyde as a pulpotomy agent in humans that it is currently impossible to assess its effectiveness. In view of these concerns relating to formocresol and the need to evaluate alternative pulpotomy agents, this study was undertaken to investigate the systemic absorption of glutaraldehyde from vital pulpotomy sites.

Methods and Materials

Dogs weighing 20–25 kg were anesthetized with pentobarbital sodium (30 mg/kg). Polyethylene catheters were placed in the femoral artery for collecting blood samples and in the femoral vein for the infusion of 5% mannitol to facilitate urine collections. A Foley catheter[a] was placed in the bladder for collection of timed urine samples. A cuffed endotracheal tube[b] (10 mm ID) was placed and connected via a one-way expirating valve to a heavy-walled, 120-liter bag for the collection of all expired air. Pulpotomies were performed on the 16 maxillary and mandibular anterior teeth of each animal. Pulpal hemostasis was obtained with cotton pellets and control samples of blood, urine, and expired air were obtained. One cotton pellet containing 20μl of 2.5% ^{14}C-(1,5) glutaral-

[a] 12 French — Inmed Corp: Norcross, GA.
[b] Curity — The Kendall Co: Boston, MA.

dehyde[c] with an activity of 6.25×10^5 dm/μl was placed in each pulpotomy site (specific activity 2.28 mCi/mole). Concetrated stock solutions of glutaraldehyde may contain a variety of molecules other than the glutaraldehyde monomer, and buffered dilute solutions should be kept in cold storage.[23] The ^{14}C-glutaraldehyde (5.0%) was diluted just before use with an equal volume of 50 mM phosphate buffer to bring the pH to 7.4 on the day of the experiment. ^{14}C is a radioactive tracer used for identification and quantification of the glutaraldehyde absorption and metabolism. After 5 min the cotton pellets were removed. Whole blood samples were collected at 15-min intervals through 90 min. Urine collections were made at 0–30, 30–60, and 60–90 min.

Expired air was bubbled slowly through 2 carbon dioxide traps connected in series, each containing 50 cc of Hyamine hydroxide.[c] Aliquots of these traps were counted in a liquid scintillation counter.[d]

The animals were sacrificed and tissue samples removed from the liver, kidney, lung, heart, and diaphragm. Bile samples also were collected. These samples were weighed, homogenized in a Polytron[e] at a tissue-to-water ratio of 1:1. Aliquots of the homogenate were placed in liquid scintillation vials and decolorized with 30% H_2O_2 prior to liquid scintillation counting. All samples were counted repeatedly until stable counts were obtained. At least 10,000 counts were collected for each sample. Quench corrections were made using external standards and the data expressed as dpm. Plasma samples were acidified and purged with N_2 to remove all traces of $^{14}CO_2$.

In separate experiments, several dogs received pulpotomy treatments consisting of saline, undiluted formocresol, 2.5% nonradioactive glutaraldehyde (pH 7.4) or 50% nonradioactive glutaraldehyde (pH 3.5). Five minutes later the cotton pellets containing the various treatment agents were removed and replaced with cotton pellets containing 20 μl of ^{125}I at pH 7.4 at an activity of 1.5×10^6 cpm/μl (1 mM). The time course of the appearance of ^{125}I in plasma was quantitated, as was the volume of distribution of iodide, to permit quantitation of total ^{125}I absorption from each pulpotomy site. This was done to evaluate the functional properties of local pulpal circulation beneath the pulpotomy treatment.

Autoradiographic specimens were prepared to grossly localize the ^{14}C-glutaraldehyde within the tooth and surrounding tissue. The anterior portion of the mandible was removed from 2 dogs with a high-speed handpiece. The sections were embedded in methyl methacrylate and sectioned with a thin sectioning machine[f] into 150 μm-thick horizontal and cross sections. These sections were placed between 2 pieces of dental x-ray film in a cassette. Two weeks later the film was processed to localize the ^{14}C-activity.

[c] Custom synthesis — New England Nuclear: Boston, MA.
[d] Model LS 3801 — Beckman Instruments: Irving, CA.
[e] Brinkman Instruments: Westbury, NY.
[f] Gillings-Hamco-Bronwill Scientific Co: Rochester, NY.

Results

Figure 1 shows the time course of the appearance of ^{14}C-activity in the plasma and urine of 5 dogs after

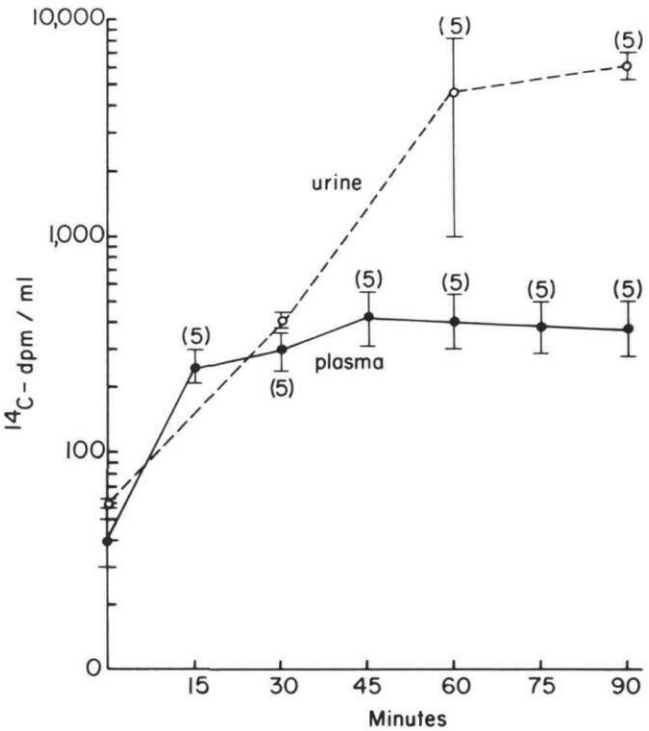

Fig 1. Time course of the appearance of ^{14}C-activity in plasma and urine after ^{14}C-glutaraldehyde application to vital pulp tissue.

Fig 2. Longitudinal section through the roots of anterior teeth treated with ^{14}C-glutaraldehyde as a pulpotomy agent.

applying radioactive 2.5% glutaraldehyde to each of 16 pulpotomy sites for 5 min. The mean plasma ^{14}C-activity level peaked in the plasma at 45 min, then began a gradual decline. The urine ^{14}C-activity continued to increase throughout the experimental period, but was always ~10 × higher than that of plasma collected at the same time. The total absorption of ^{14}C-glutaraldehyde in 5 dogs is shown in Table 1. The systemic absorption was 2.91 ± 0.94% (N=5) of the total applied dose or 9.32 ± 3.02μl (N=5)/60 min.

The percentage distribution of absorbed ^{14}C-glutaraldehyde excreted in bile, urine, and exhaled as CO_2 is shown in Table 2. Approximately 0.6% of the absorbed dose was found in bile. Urinary excretion accounted for about 8% of the absorbed dose with pulmonary excretion accounting for almost 4%. The urinary and pulmonary excretions of ^{14}C-glutaraldehyde were similar to those found with ^{14}C-formaldehyde, but the biliary excretion of ^{14}C-glutaraldehyde was much less than that of ^{14}C-formaldehyde.[8]

Table 3 shows the ratios of ^{14}C-activity in various tissues compared to the 60-min plasma values. The highest T/P ratio for ^{14}C-glutaraldehyde was 2.21 for red blood cells. However, most tissues had T/P ratios that were not statistically different from 1.0 ($\bar{x}$ = 1.24 ± 0.39, N = 8) suggesting that ^{14}C-glutaraldehyde, at the tracer concentrations present in plasma, did not show high binding capacities. The volume of distribution of ^{14}C-glutaraldehyde was 79.4 ± 9.1% (N = 5) μl body weight.

Table 4 shows the level of ^{125}I-activity in plasma following application of the isotope to formocresol- or glutaraldehyde-treated pulpotomy sites and to a normal saline-treated pulpotomy site. Relatively little ^{125}I was absorbed following treatment of the pulpotomy site with either 50% glutaraldehyde or formocresol (19% formaldehyde, 35% cresol). The ^{125}I-activity in the plasma was nearly 3 times higher following ^{125}I application to a pulpotomy site treated with 2.5% glutaraldehyde compared to a formocresol-treated site. The ^{125}I-activity increased nearly 4 times above a formocresol-treated site after application of ^{125}I to a saline-treated pulpotomy site. Application of 50% glutaraldehyde to a pulpotomy site decreased the absorption of ^{125}I from that site below that seen with formocresol (Table 4) and almost 5 times compared to a pulpotomy treated with 2.5% glutaraldehyde. The site treated with 2.5% glutaraldehyde absorbed almost as much ^{125}I as pulpotomy sites treated with isotonic saline (Table 4).

Figure 2 represents a longitudinal section and Figure 3 a cross section through the roots of anterior teeth treated with ^{14}C-gluraraldehyde. Note that the radioactive material (black) clearly outlined the pulp canals and roots and was confined essentially to the pulp canals and roots of the tooth with only traces of radioactive material visible in the periodontal membrane space and surrounding bone.

Discussion

The results of this study demonstrate that systemic absorption of glutaraldehyde occurs following 5-min applications of 2% glutaraldehyde to pulpotomy sites. The absorption peaked at 45 min after application to the pulpotomy sites, indicating that absorption continued for some time after the glutaraldehyde pellets were removed. This absorption pattern is somewhat different from that reported in the authors' previous work on formocresol wherein the absorption appeared to be quickly self-limiting.[7,8] When formocresol pellets were sealed in the teeth, the absorption peaked in 15–30 min and then began to decline. Apparently, a 2% concentration of glutaraldehyde is less damaging to the microcirculation of the pulp, producing a more gradual impairment of the microcirculation than does undiluted formocresol. The appearance of ^{14}C-activity in the urine indicates that a significant fraction of the absorbed glutaraldehyde or its metabolites, was freely filterable in the kidney.

The biliary renal and pulmonary excretion of ^{14}C-activity indicates that a small but significant fraction of the ^{14}C-glutaraldehyde that was absorbed, subsequently was excreted. The actual chemical form of the

Table 1. Total Absorption of ^{14}C-Glutaraldehyde

Dog #	Absorption (dpm)*	% Dose**	μl/hr***
1	2.70×10^6	1.35	4.32
2	2.87×10^6	1.44	4.59
3	4.05×10^6	2.03	6.48
4	6.70×10^6	3.35	10.72
5	1.28×10^7	6.40	20.48
$\bar{x}$ ± SEM	$3.29 \times 10^6 \pm 1.07 \times 10^6$	2.91 ± 0.94	9.32 ± 3.02

* Total absorption determined by multiplying peak plasma value by the subsequently determined volume of distribution of ^{14}C-glutaraldehyde in each dog.

** Dose was 2×10^8 dpm on 16 pellets containing 20 μl, 2% ^{14}C-glutaraldehyde.

*** Calculated based on applied concentration of 6.25×10^5 dpm/μl.

Table 2. Relative Distribution of ^{14}C-Glutaraldehyde Activity That Was Absorbed

Excretory Distribution		*% Absorbed Dose*
Biliary	$3.98 \times 10^5 \pm 3.48 \times 10^4$(2) dpm	0.60 ± 0.24(2)
Urinary	$9.73 \times 10^5 \pm 2.87 \times 10^5$(2) dpm	8.16 ± 0.40(2)
Pulmonary	$4.12 \times 10^5 \pm 9.05 \times 10^4$(2) dpm	3.60 ± 0.03(2)
Tissue Distribution		
Liver	$2.07 \times 10^5 \pm 3.85 \times 10^4$(2) dpm	1.82 ± 0.08(2)
Muscle	$5.53 \times 10^6 \pm 1.31 \times 10^6$(2) dpm	51.16 ± 2.31(2)
Plasma	$5.51 \times 10^5 \pm 9.65 \times 10^4$(2) dpm	4.88 ± 0.26(2)
RBC	$1.07 \times 10^6 \pm 3.64 \times 10^5$(2) dpm	11.81 ± 1.60(2)
Heart	$9.46 \times 10^4 \pm 1.74 \times 10^4$(2) dpm	0.83 ± 0.03(2)
Lung	$1.62 \times 10^5 \pm 2.95 \times 10^4$(2) dpm	1.43 ± 0.06(2)
Spleen	$1.39 \times 10^4 \pm 2.5 \times 10^3$(2) dpm	0.12 ± 0.05(2)
Kidney	$1.30 \times 10^5 \pm 2.35 \times 10^4$(2) dpm	1.06 ± 0.03(2)
Total	9.54×10^6 dpm	85.47 ± 5.09%

Table 3. Tissue-to-Plasma ^{14}C-Glutaraldehyde Activity Ratios

	*T/P**
Liver	0.47 ± 0.06(5)
Lung	1.51 ± 0.55(5)
Muscle	1.11 ± 0.79(6)
Heart	0.96 ± 0.30(4)
Spleen	0.52 ± 0.24(4)
Kidney	—
cortex	1.64 ± 0.12(5)
medulla	1.56 ± 0.14(3)
RBC	2.21 ± 0.92(2)

*Tissue-to-plasma ratios (T/P) expressed as the activity of ^{14}C-glutaraldehyde/gm of tissue or plasma water.

Table 4. Systemic Absorption of ^{125}I from Pulpotomy Sites

Pulpotomy Agent	*% Dose Absorbed*	*µl/60 Min Absorbed*
Undiluted formocresol	6.5 ± 4.2(2)	0.7 ± 0.5(2)
Isotonic saline	23.9 ± 5.8(2)	2.5 ± 0.6(2)
50% glutaraldehyde	3.4(1)	0.3(1)
2.5% glutaraldehyde	16.4(1)	1.6(1)

^{14}C-activity was unknown, although that activity exhaled from the lungs was in the form of $^{14}CO_2$, indicating that at least some of the glutaraldehyde had been decarboxylated.

The tissue-to-plasma ^{14}C-activity ratios of the various tissues indicated that, with the exception of erythrocytes, there was little tissue binding of ^{14}C-activity. A ratio of 1.0 indicates that the ^{14}C-glutaraldehyde activity in plasma and interstitial fluid water had equilibrated with the tissue cell water and there was no tissue binding. Ratios less than 1.0 indicate that the ^{14}C-activity did not equilibrate completely with the tissue water. Ratios greater than 1.0 indicate tissue binding since there was more ^{14}C-activity than could be accounted for by equilibration between

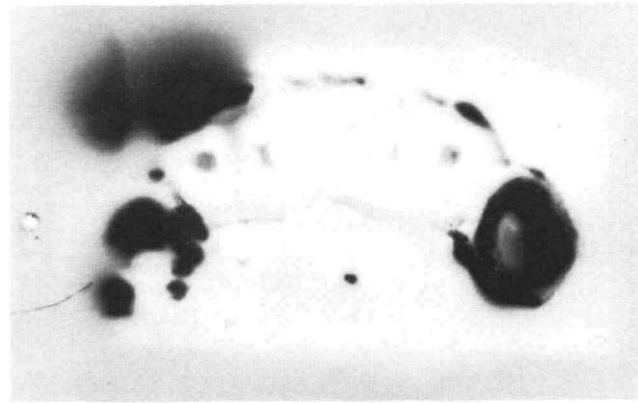

Fig 3. Cross section through the roots of anterior teeth treated with ^{14}C-glutaraldehyde as a pulpotomy agent.

plasma and tissue water. The volume of distribution of ^{14}C-glutaraldehyde (79.4% of body weight) also indicates little binding. That is, ^{14}C-glutaraldehyde did not have a volume of distribution that was much larger than the total body water of the animals (67% of body weight). This was in marked contrast to the volume of distribution of ^{14}C-formaldehyde of 129% of body weight that was reported previously, a value about twice that of total body water, and a mean T/P ratio for ^{14}C-formaldehyde of 2.21.[8]

The ^{125}I uptake data also suggest that only limited impairment of the microcirculation occurred following the application of 2% glutaraldehyde to the pulpotomy site compared to the injury resulting from standard formocresol.[7] Although the absolute value of the absorbed dose of glutaraldehyde is low (2.91 ± 0.94%, N = 5) compared to formocresol (7.30 ± 2.37%, N = 2) reported in the previous investigation, the values are not statistically different ($p < 0.1$).[8] The systemic absorptions of glutaraldehyde and formocresol can be compared as pulpal clearance values (µl/60 min), to correct for differences in applied concentrations (Table 1).

Comparison of the pulpal clearance values indicates that the systemic absorption of ^{14}C-glutaraldehyde (9.32 ± 3.02 µl/hr from Table 1) and ^{14}C-formaldehyde (11.53 ± 3.52 µl/hr recalculated from a previous experiment[8]) are very similar. The concentration of glutaraldehyde used in this study was only 2.5% or 0.25 M in contrast to the concentration of formaldehyde (19% or 6.3 M) used in formocresol. Investigations using higher concentrations of glutaraldehyde might show a larger amount of glutaraldehyde absorbed systemically.

Conclusions

The results of this study demonstrate that 2.5% glutaraldehyde is absorbed from vital pulpotomy sites.

The tissue-to-plasma ratios and the volume of distribution were lower than those obtained in the previous experiments with formocresol, indicating that not only was less glutaraldehyde absorbed but less of the absorbed glutaraldehyde was bound in the tissues. The autoradiographs indicate that the absorbed glutaraldehyde was limited largely to the pulp space with little evidence of glutaraldehyde outside the tooth. Results of this study alone do not demonstrate that glutaraldehyde is a satisfactory pulpotomy agent.

Supported by NIDR Grants DE07013 and DE06427.

A portion of this manuscript was presented at the annual meeting of the American Association for Dental Research, Washington, DC, March, 1985.

Dr. Myers is a professor and chairman, pedodontics; Dr. Pashley is a professor, oral biology/physiology; Dr. Lake is an associate professor, oral biology/anatomy; Mr. Burnham and Ms. Kalathoor are research assistants, oral biology/physiology; and Mr. Waters is a senior dental student and AADR Student Research Fellow, School of Dentistry, Medical College of Georgia, Augusta, Georgia. Reprint requests should be sent to: Dr. David R. Myers, Dept. of Pedodontics, School of Dentistry, Medical College of Georgia, Augusta, GA 30912.

1. Rolling I, Lambjerg-Hansen H: Pulp condition of successfully formocresol-treated primary molars. Scand J Dent Res 86:267–72, 1978.
2. Rolling I, Hasselgren E, Tronstad L: Morphologic and enzyme histochemical observations on the pulp of human primary molars three to five years after formocresol treatment. Oral Surg 42:518–28, 1976.
3. Magnusson BO: Therapeutic pulpotomies in primary molars with the formocresol technique: a clinical and histological follow up. Acta Odontol Scand 36:157–65, 1978.
4. Block RM, Lewis RD, Sheats JB, Fauley J: Cell-mediated immune response to dog pulp tissue altered by formocresol within the root canal. J Endod 3:424–30, 1977.
5. 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.
6. Pruhs RJ, Olen GA, Sharma PS: Relationship between formocresol pulpotomies on primary teeth and enamel defects on their permanent successors. J Am Dent Assoc 94:698–700, 1977.
7. Myers DR, Shoaf HK, Dirksen TR, Pashley DH, Whitford GM, Reynolds KE: Distribution of ^{14}C-formaldehyde after pulpotomy with formocresol. J Am Dent Assoc 96:805–13, 1978.
8. Pashley EL, Myers DR, Pashley DH, Whitford GM: Systemic distribution of ^{14}C-formaldehyde from formocresol-treated pulpotomy sites. J Dent Res 49:602–8, 1980.
9. Myers DR, Pashley DH, Whitford GM, Sobel RE, McKinney RV: The acute toxicity of systemically administered formocresol in dogs. Pediatr Dent 3:37–41, 1981.
10. 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.
11. s'Gravenmeade EJ: Some biochemical considerations of fixation in endodontics. J Endod 1:233–37, 1975.
12. Davis MJ, Myers R, Switkes MD: Glutaraldehyde: an alternative to formocresol for vital pulp therapy. J Dent Child 49:176–80, 1982.
13. Dawes CJ: Biological Techniques for Transmission and Scanning Electron Microscopy. Burlington, Vermont: Ladd Research Industries Inc, 1979 p 52.
14. Cunningham KW, Lazzari EP, Ranly DM: The effect of formocresol and glutaraldehyde on certain enzymes in bovine dental pulp. Oral Surg 54:100–103, 1982.
15. Ranly DM, Lazzari EP: A biochemical study of two bifunctional reagents as alternatives to formocresol. J Dent Res 62:1054–57, 1983.
16. Ranly DM: The effect of pH and concentration on the glutaraldehyde fixation of bovine pulp: an in vitro study. Acta Odontol Pediatr 4:45–47, 1983.
17. 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.
18. Wemes JC, Jansen HWB, Purdell-Lewis D, Boering G: Histologic evaluation of the effect of formocresol and glutaraldehyde on the periapical tissues after endodontic treatment. Oral Surg 54:329–32, 1982.
19. Lekka M, Hume WR, Wolinsky LE: Comparison between formaldehyde and glutaraldehyde diffusion through the root tissues of pulpotomy-treated teeth. J Pedod 8:185–91, 1984.
20. Ranly DM, Garcia-Godoy F: The diffusion of glutaraldehyde from zinc oxide-eugenol cement. Pediatr Dent 7:215–17, 1985.
21. Kopel HM, Bernick S, Zachrisson E, DeRomero SA: The effect of glutaraldehyde on primary tissue following coronal amputation: an in vivo histologic study. J Dent Child 47:425–30, 1980.
22. Garcia-Godoy F: Clinical evaluation of glutaraldehyde pulpotomies in primary teeth. Acta Odontol Pediatr 4:41–44, 1983.
23. Ranly DM: Glutaraldehyde purity and stability: implications for preparation, storage, and use as a pulpotomy agent. Pediatr Dent 6:83–87, 1984.