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The oxidation of glutaraldehyde by rat tissues

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Abstract

Glutaraldehyde is used in pediatric dentistry as an alternative to formocresol in the treatment of primary teeth with diseased pulpal tissue. Because of concerns about the effects of glutaraldehyde in biological systems, the metabolism of ^{14}C-(1, 5) glutaraldehyde (2.28 mC/mmole) in rat tissues was investigated. Tissue slices prepared from liver, heart, kidney, and muscle were incubated in Krebs-Ringer bicarbonate buffer in the presence of ^{14}C-glutaraldehyde (800,000 DPM); the $^{14}CO_2$ was measured. The DPM $^{14}CO_2$ (mean ± SD) produced/400 mg slices (wet weight)/2 hr in liver, heart, kidney, and muscle were 16,385 ± 3124, 5000 ± 1217, 38,622 ± 6233, and 1774 ± 265, respectively. A 10,000 g "mitochondrial" subfraction was prepared from kidney and incubated with glutaraldehyde (100,000 DPM). The production of $^{14}CO_2$ was linear for 15 min and up to 5 mg protein. It is concluded that glutaraldehyde can be metabolized in living systems.

Concern over the clinical use of formocresol as a pulpal fixative stems from doubts about its clinical effectiveness (Magnusson 1978), local irritating effects (Block et al. 1978), systemic absorption (Pashley et al. 1980), and toxicity (Lewis and Chestner 1981). Glutaraldehyde has been suggested as the potential pulpotomy agent of choice to replace formocresol (Davis et al. 1982) in the dental armamentarium. A popular dentin primer called Gluma, which is used prior to composite bonding, contains 5% glutaraldehyde (Munksgaard et al. 1985). Although glutaraldehyde, a bifunctional reagent, is an effective protein cross-linking agent and, therefore, a powerful fixative (Dawes 1979; Ranly and Lazzari 1983), little information is available concerning the metabolism of this compound in the body (Horton 1977). This paper presents data on the metabolism of glutaraldehyde in rat tissue.

Materials and Methods

Chemicals

^{14}C- (1, 5) glutaraldehyde (specific activity, 2.28 mCi/mmole) was synthesized by New England Nuclear, Boston, Massachusetts. DL-isocitrate, trisodium salt, alpha-ketoglutarate, disodium salt, glutaric acid, and human serum albumin (free fatty acid free-Fraction V) were purchased from Sigma Chemical Co., St. Louis, Missouri. ReadySolv HP was purchased from SmithKline/Beckman, Irvine, California. All other chemicals were reagent grade.

Tissue Preparation

Adult, ad libitum-fed Fisher 344 inbred rats were sacrificed by decapitation and kidney, liver, muscle, and hearts collected and immediately cooled on ice. All subsequent procedures were performed at 0-4°C. For the slice experiments, four rats were sacrificed simultaneously and slices made from the liver, gastrocnemius muscle, kidney, and the heart of each animal. Slices from each specific tissue from all animals were pooled and randomized by stirring gently. For preparation of a "mitochondrial" fraction, kidneys were removed, weighed, and homogenized in 10 volumes 0.25 M sucrose, using a Potter-Elvejhm device. The whole homogenate was centrifuged at 600 g for 10 min and the supernatant recentrifuged at 10,000 g for another 10 min. The resulting 10,000-g pellet was labeled the mitochondrial fraction and resuspended in 1-2 ml 0.25 M sucrose.

Incubations

The pooled slices (approximately 400 mg, total wet weight) were incubated in 2 ml of Krebs-Ringer bicarbonate buffer containing about 800,000 DPM ^{14}C-glutaraldehyde. Incubations, in quadruplicate, were carried out at 37°C for 2 hr in sealed serum bottles fitted with a center well for CO_2 collection (Robertson et al. 1971); the CO_2 formed was collected in hyamine hydroxide for 30 min after the reaction was terminated with 2N H_2SO_4. The center wells filled with hyamine hydroxide were dropped into scintillation vials containing ReadySolv HP. Results are expressed as DPM ^{14}CO produced per 400 mg slices per two hr. In the control

incubations, the slices were boiled at 100°C for 10 min prior to incubation.

The incubation for the mitochondrial fractions contained MgCl2 (10 mmole/L), KCl (10 mmole/L), glucose (40 mmole/L), sodium phosphate buffer (100 mmole/L, pH 7.4), mitochondrial protein (3-4 mg), and ^{14}C-glutaraldehyde (approximately 100,000 DPM); total volume was 3 ml. Incubations were performed in triplicate and CO_2 collected and measured as described above. Results are expressed as DPM CO_2 produced per mg protein per hr. Experiments in which the mitochondrial fraction was boiled for 10 min at 100°C prior to incubation or "no-enzyme" experiments in which water was substituted for the mitochondrial fraction generally produced about 150 DPMs. The authors routinely ran no-enzyme controls and subtracted the results from the experimental incubations.

Protein was measured using the Lowry assay (Lowry et al. 1951). Human serum albumin was used as a standard. Counting efficiency was determined using an internal 14C-toluene standard and was approximately 93%; all samples were counted to within a 3% counting error.

Results

The results obtained in the studies measuring $^{14}CO_2$ production from glutaraldehyde in slices from different rat tissues are presented in Table 1. Rat kidney showed the greatest amount of $^{14}CO_2$ production, followed by liver, heart, and muscle. Control experiments in which the slices were first boiled and then incubated, routinely produced about 100 DPM CO_2.

Since kidney had the greatest ability to oxidize glutaraldehyde, kidney was used to isolate a 10,000 g mitochondrial pellet. In order to provide additional evidence that $^{14}CO_2$ is produced from glutaraldehyde enzymatically, the oxidation of glutaraldehyde in rat kidney mitochondria was studied as a function of incubation time and protein concentration. Figure 1 shows the results obtained when incubation time was varied over 2 hr. The production of $^{14}CO_2$ was linear to about 20 min and reached a plateau at 100 min. Figure 2 shows the results of varying the amount of protein in the incubation. The production of $^{14}CO_2$ was linear to about 10 mg protein/incubation and reached a plateau at approximately 14 mg protein/incubation. When the 10,000 g supernatant (4.2 mg protein) was added to incubations in lieu of mitochondrial protein, $^{14}CO_2$ production was equal to control incubations. These results are typical of processes occurring enzymatically.

TABLE 1. Glutaraldehyde Oxidation by Rat Tissue Slices

Organ	*Treatment*	*Total DPM $^{14}CO_2$*
Kidney	Experiment	38,622 ± 6233
	Control	92 11
Liver	Experiment	16,385 3124
	Control	106 5
Heart	Experiment	5000 1217
	Control	110 30
Muscle	Experiment	1774 265
	Control	97 ± 13

The experimental incubations contained approximately 400 mg (wet weight) tissue slices, 2 ml of Krebs-Ringer bicarbonate buffer, and approximately 800,000 DPM ^{14}C-glutaraldehyde (specific activity = 2.28 mCi/mmole). In the control incubations, the slices were boiled at 100°C for 10 min prior to incubation. Results are expressed as total DPM CO_2 produced per 400 mg slices per 2 hr. Mean ± standard deviation is based on quadruplicate incubations.

Table 2 (page 303) shows the results obtained when the authors measured $^{14}CO_2$ production in kidney mitochondria in the basic incubation medium and with the addition of isocitrate, alpha-ketoglutarate, or glutaric acid. In the presence of isocitrate, the production of $^{14}CO_2$ increased 510% compared to the basic incubation medium; alpha-ketoglutarate increased $^{14}CO_2$ production 631%, while the production of $^{14}CO_2$ decreased 92% in the presence of glutaric acid.

Discussion

The results demonstrate that glutaraldehyde is metabolized in rat tissues, with kidney showing the greatest activity. Furthermore, oxidation of glutaraldehyde occurs in the mitochondria fraction, not the 10,000 g supernatant fraction. The data are consistent with the observations of Myers et al. (1986) who reported the

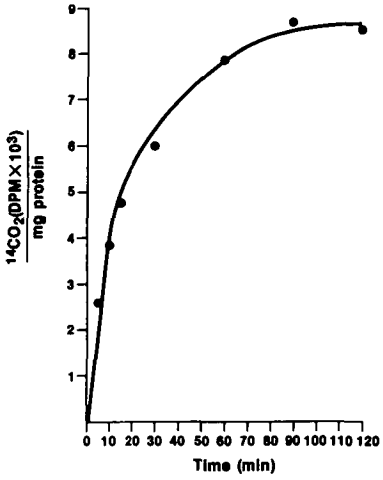

FIG 1. Glutaraldehyde oxidation by rat kidney "mitochondria" as a function of incubation time. The basic incubation medium additionally contained 5 mmole/L dl-isocitrate. Points on the curve represent the mean of triplicate determinations.

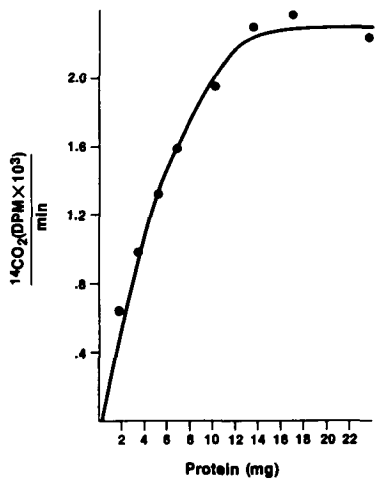

FIG 2. Glutaraldehyde oxidation by rat kidney "mitochondria" as a function of protein concentration. The basic incubation medium additionally contained 5 mmole/L dl-isocitrate. Points on the curve represent the mean of triplicate determinations.

TABLE 2. Glutaraldehyde Oxidation by Rat Kidney "Mitochondria"

Experiment	*DPM CO_2/ mg/hr*	*% Change*
Basic incubation	999 ± 416	—
Basic incubation + DL-isocitrate	6094 834	+ 510
Basic incubation + ketoglutarate	7298 454	+ 631
Basic incubation + glutaric acid	77 ± 67	− 92

The basic incubation contained $MgCl_2$ (10 mmole/liter), KCl (10 mmole/liter), glucose (40 mmole/liter), sodium phosphate buffer (100 mmole/liter, pH 7.4), mitochondrial protein (3–4 mg), and approximately 100,000 DPM ^{14}C-glutaraldehyde (specific activity = 2.28 mCi/mmole). DL-isocitrate, alpha-ketoglutarate or glutaric acid were added to the basic incubation at a concentration of 2 mmole/liter. Mean ± standard deviation is based on triplicate determinations.

detection of radioactive CO_2 in the expired air of dogs on which pulpotomies had been performed with ^{14}C-glutaraldehyde. Comparing the metabolism of glutaraldehyde with formaldehyde, previous work has shown that formocresol is absorbed and distributed rapidly throughout the body within minutes of being placed on a pulpotomy site. In addition, only a very small fraction of formaldehyde is metabolized to $^{14}CO_2$; most formaldehyde is tissue bound (Pashley et al. 1980). In contrast, glutaraldehyde has a very low tissue binding (Myers et al. 1986) and is readily metabolized. These observations support the replacement of formaldehyde with glutaraldehyde as a pulpotomy agent for primary teeth.

It is possible that glutaraldehyde may be metabolized similarly to other aldehydes, such as acetaldehyde. Much research has centered on acetaldehyde metabolism (Thurman 1980), because of interest in this aldehyde as an intermediate in alcohol metabolism. Whether glutaraldehyde is acted upon by the same enzymes that metabolize acetaldehyde, or more specific enzymes, possibly glutaraldehyde dehydrogenases and semialdehyde dehydrogenases, needs to be investigated.

The increase in the amount of $^{14}CO_2$ produced in the presence of alpha-ketoglutarate is approximately equal to the increase when dl-isocitrate is added (Table 2). This may indicate that both alpha-ketoglutarate and dl-isocitrate stimulated $^{14}CO_2$ by stimulating the tricarboxylic acid cycle. The decrease in $^{14}CO_2$ produced in the presence of glutaric acid may be due to an isotope-dilution effect or the inhibition of glutaraldehyde oxidation by glutaric acid. Further study is needed to clarify the role of alpha-ketoglutarate, dl-isocitrate, and glutaric acid in glutaraldehyde oxidation.

These data are considered important because they illustrate that a tissue fixative presently being used in dentistry, glutaraldehyde, can be metabolized in rat tissue. The level at which glutaraldehyde ceases to be a dental tissue fixative and instead becomes a metabolite needs to be investigated.

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