Distribution and Excretion of TEGDMA in Guinea Pigs and Mice

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ABSTRACT

The monomer triethyleneglycoldimethacrylate (TEGDMA) is used as a diluent in many resin-based dental materials. It was previously shown *in vitro* that TEGDMA was released into the adjacent biophase from such materials during the first days after placement. In this study, the uptake, distribution, and excretion of ^{14}C-TEGDMA applied *via* gastric, intradermal, and intravenous administration at dose levels well above those encountered in dental care were examined in vivo in guinea pigs and mice as a test of the hypothesis that TEGDMA reaches cytotoxic levels in mammalian tissues. ^{14}C-TEGDMA was taken up rapidly from the stomach and small intestine after gastric administration in both species and was widely distributed in the body following administration by each route. Most ^{14}C was excreted within one day as $^{14}CO_2$. The peak equivalent TEGDMA levels in all mouse and guinea pig tissues examined were at least 1000-fold less than known toxic levels. The study therefore did not support the hypothesis.

KEY WORDS: TEGDMA, composite resin, restorative materials, toxicity.

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INTRODUCTION

Resin-containing materials are used routinely in dental practice as direct filling materials, fissure sealing agents, bonding resins, and resin cements. Among the components of most bonding and restorative resins are (1) a primary resin, usually 2,2-bis-(4-(2-hydroxy-3-methacryloxy-propoxy)phenyl)propane (Bis-GMA), and (2) triethyleneglycol-dimethacrylate (TEGDMA), which is included to compensate for the high viscosity of the primary resin. Resin composites contain TEGDMA in amounts from 15 to 25%, while bonding resins contain TEGDMA in the range 30 to 55% (Nakabayashi and Takarada, 1992).

Direct evidence of TEGDMA release from composite resins and fissure sealants into the biophase was provided by Tanaka *et al.* (1991), Gerzina and Hume (1994), Hamid and Hume (1997), and Spahl *et al.* (1998). TEGDMA can be expected to enter the body by two different routes after resin placement: *via* the saliva and gastrointestinal tract, plus (if the material is placed onto dentin) *via* the dentin and pulp (Hume and Gerzina, 1996).

To test the hypothesis that TEGDMA reaches cytotoxic levels in body tissues, we have measured the uptake, distribution, and clearance of ^{14}C-TEGDMA administered by gastric tube and by subcutaneous injection in guinea pigs and by the same routes and by intravenous injection in mice.

MATERIALS & METHODS

^{14}C-TEGDMA was purchased from Prins-Maurits-Laboratorium (Rijswijk, The Netherlands), dissolved in dichloromethane, and stored at -20°C. Unlabeled TEGDMA was obtained from ESPE Dental AG (Seefeld, Germany).

Guinea Pigs

The University of Munich Committee on Animal Research, ensuring humane practices, approved the experiments (permission no. 211-2531-66/94). Adult male guinea pigs (Dunkin-Hartley Pirbright white strain) were fed a standard diet and water *ad libitum*. Sixteen guinea pigs were allotted to 4 groups of 4 animals each. Each animal was put into a separate metabolic cage 3 days before and food was removed 12 hrs before the experiment. Each animal received 0.02 mmol/kg ^{14}C-TEGDMA (0.7 kBq/g) either by subcutaneous injection (group 1) or *via* gastric tube (group 2). Control animals (groups 3 and 4) received 0.9% NaCl solution correspondingly. Feces and urine were collected at 1, 2, 4, 6, 8, 12, and 24 hrs after ^{14}C-TEGDMA administration, and the ^{14}C-radioactivity was measured as described below. At 24 hrs, the animals were killed in ether. Organs taken immediately and tested were: liver, kidney, blood, skin, brain, heart, spleen, lung, muscle, testes, eyes, bone, nerve tissue, spinal cord, wall of stomach, content of stomach, wall of ileum + jejunum, content of ileum + jejunum, wall of colon, content of colon, wall of caecum, content of caecum, wall of gall bladder, and fat

tissue. Organs were immediately washed with 2 x 10 mL distilled H_2O, with the wash-water saved, and then the tissues were weighed and homogenized. Tissues were dissolved in tetraethylammoniumhydroxide (TEAH) (20%) in aqueous solution with Omni-Szintisol® (both from Merck, Darmstadt, Germany). ^{14}C was determined with a liquid scintillation counter (2500 TR, Canberra-Packard, Dreieich, Germany). Data were presented as mean ± standard error of the mean (SEM) of the administered dose. Statistical significance of differences between experimental groups was determined by means of the Bonferroni-Holm *t* test (Forst, 1985).

A second set of 16 guinea pigs was treated as described above, with the addition that each animal was kept in a closed chamber with controlled airflow. The exhaled air was captured during the 24-hour experimental period by flowing through 7 bottles, one behind the other, filled with 250 mL ice-cold 5 N NaOH (see Fig.). $^{14}CO_2$ was captured as $Na_2{}^{14}CO_3$, and the total ^{14}C activity was determined.

Mice

The UCLA Committee on Animal Research, ensuring humane practices, approved the experiments. Twenty-three Balb-C, female mice, 6 to 7 weeks of age, were obtained from the UCLA animal colony and housed and fed routinely before the administration of 10 nanomoles (50 nCi total activity, 100 nmol/mL, 0.1 mL administered volume) ^{14}C-TEGDMA by one of three routes: (1) by gastric tube, (2) into the tail vein, or (3) by intradermal injection beneath shoulder skin. Animals were killed by decapitation at various times after administration (see Tables 2 and 3), and samples of blood and various tissues (see Tables) were taken. Tissues, blood, and feces were weighed then dissolved in Hionic-Fluor (Packard, Meridien, CT, USA) and urine samples in Ultima Gold LLT (Packard), and ^{14}C was determined by means of a liquid scintillation counter (LS6500, Beckman Coulter, Fullerton, CA, USA). Data were expressed as femtomoles/mg TEGDMA on the assumption that ^{14}C represented ^{14}C-TEGDMA.

RESULTS

Total ^{14}C Recovery from Guinea Pigs after 24 Hrs

Table 1 summarizes data for ^{14}C excretion in guinea pigs *via* the urine, feces, and in exhaled carbon dioxide during 24 hrs after ^{14}C-TEGDMA administration and ^{14}C distribution in the organs collected at the time the guinea pigs were killed, expressed as a percentage of the ^{14}C-TEGDMA dose administered. During the first 24 hrs after administration, guinea pigs exhaled $^{14}CO_2$, equivalent to about 60% of the ^{14}C-TEGDMA administered with each route. About 15% was excreted in the urine and about 5% remained in the tissues at 24 hrs. The total ^{14}C recovery was more than 80% of the ^{14}C-TEGDMA dose administered.

Table 1. ^{14}C Excretion and Summed ^{14}C Distribution in Guinea Pigs

	Percentage of the ^{14}C-TEGDMA Dose Administered[a]			
	Subcutaneous		Gastric Tube	
	Mean	SEM	Mean	SEM
Urine	16.5	1.2	15.1	3.6
Feces	0.3	0.1	0.5	0.1
Exhaled $^{14}CO_2$	63.6	2.1	61.9	4.6
Organ wash-water	0.5	0.1	0.5	0.1
Summed organs	5.0	1.2	5.2	1.0
Total ^{14}C recovery	85.9	4.9	83.2	4.1

[a] ^{14}C excretion in guinea pigs *via* the urine, feces, and carbon dioxide and summed ^{14}C distribution in all organs 24 hrs after administration, expressed as a percentage of the administered dose.

Mouse-Gastric Administration

Table 2 shows the amounts of ^{14}C expressed as the equivalent of TEGDMA *per* milligram in tissue samples from each of 7 animals which were killed at the times shown after administration of ^{14}C-TEGDMA (10 nanomoles total dose) by gastric tube. Virtually all detectable ^{14}C was cleared from the mice in one day.

Mouse-Intravenous Administration

Table 3 shows the amounts of ^{14}C expressed as the equivalent of TEGDMA *per* milligram in tissue samples from each of 8 animals which were killed at the times shown after administration of ^{14}C-TEGDMA (10 nanomoles total dose) by intravenous injection. Trace amounts of ^{14}C were still present in the tissues tested after 2 days.

Mouse-Intradermal Administratn

Distribution and clearance of ^{14}C were similar with this route of administration to that with gastric administration. Virtually all detectable ^{14}C was cleared by one day after administration.

Table 2. Distribution of ^{14}C-TEGDMA Over Time Following Gastric Administration in Mice

	Time after Administration						
Tissue	1 min	15 min	30 min	1 hr	3 hrs	1 day	2 days
Stomach[a]	11699[b]	2828	372	804	359	4	0
Blood	68	119	42	64	13	0	0
Brain	9	45	27	4	0	0	0
Heart	173	98	40	8	0	0	0
Intestine[a]	260	609	229	2	8	0	0
Kidney	74	342	213	63	26	0	0
Liver	530	271	175	15	9	0	0
Lung	85	100	46	11	0	0	0
Lymph node	16	221	50	18	32	0	0
Muscle	114	111	62	23	12	0	0
Spleen	104	118	60	46	5	0	0
Thymus	35	116	21	45	7	0	0

[a] Including contents.
[b] ^{14}C expressed as the equivalent concentration of TEGDMA (femtomoles/mg) present in various tissues in each of 7 mice killed at the times shown following administration of ^{14}C-TEGDMA (10 nanomoles total dose) by gastric tube.

Table 3. Distribution of ^{14}C-TEGDMA Over Time Following Intravenous Administration in Mice

	Time after Administration							
Tissue	5 min	15 min	30 min	1 hr	3 hrs	6 hrs	1 day	2 days
Blood	280[a]	128	136	46	23	17	8	5
Brain	620	429	474	502	168	84	8	1
Heart	180	91	74	36	33	33	20	3
Intestine[b]	34	17	19	33	16	6	2	2
Kidney	412	531	400	140	68	26	9	2
Liver	65	41	57	23	12	7	3	3
Lung	228	172	154	78	45	30	12	9
Lymph node	293	146	59	110	40	61	48	10
Muscle	140	103	44	44	43	14	8	0
Spleen	179	117	90	77	46	26	18	2
Stomach[b]	-	48	37	55	42	18	13	1
Thymus	125	30	42	106	91	48	41	14

[a] ^{14}C expressed as the equivalent concentration of TEGDMA (femtomoles/mg) present in various tissues in each of 8 mice killed at the times shown following administration of ^{14}C-TEGDMA (10 nanomoles total dose) by injection into the tail vein.

[b] Including contents.

DISCUSSION

The administered dose levels were chosen to exceed substantially the body-weight-adjusted dose levels relative to humans for TEGDMA released from composite resin restorations. For guinea pigs, we used the data of Spahl *et al.* (1998), who showed that the commercial composite Superlux® provided 1.4 mmoles TEGDMA from 100 g of composite, an amount that would be used when many teeth are restored simultaneously. For the mouse, we used the data of Gerzina and Hume (1996), who showed that on the order of 0.5 µmoles of TEGDMA was released from each restoration when the commercial composite Z100® (3M) was used in human molar teeth. Despite the resultant difference in administered dose levels (20 µmol/kg for guinea pigs *vs.* 0.5 µmol/kg for mice), the data on distribution and clearance for the two species were very similar.

The body-weight-adjusted dose of TEDGMA administered was more than 50 times higher than that which a human dental patient would receive. However, the highest levels of TEGDMA observed in tissue samples taken from mice—53 nM in liver 1 min after gastric placement and 63 nM in brain 5 min after intravenous injection—are approximately 10,000-fold less than the known toxic level for TEGDMA (Hanks *et al.*, 1991; Saygili *et al.*, 1992; Reichl *et al.*, 1999a). Similarly, the highest ^{14}C concentration in the spontaneous urine in guinea pigs was found to be 0.2 mmol/L TEGDMA equivalent, 4 hrs after the subcutaneous ^{14}C application. Peak levels in blood and kidney tissue in mice occurred much earlier than this (see Tables 2 and 3). This is consistent with the known time dynamics of urine production and the variable but expected delay before urine release. The peak urinary level observed was about one-tenth of the concentration that depressed gluconeogenesis in kidney cells (Reichl *et al.*, 1999b).

Brain levels of ^{14}C following administration by gastric tube appeared to be markedly lower than those of other tissues, indicating that ^{14}C-TEGDMA administered by that route did not cross the blood-brain barrier well. The major route of uptake in humans following filling placement is very likely to be *via* the saliva and stomach. Following intravenous administration, brain levels were initially higher than those in blood and most other tissues, indicating selective uptake across the blood-brain barrier. However, the peak concentrations observed were far below any known toxic level. There is no known equivalent to intravenous application in clinical use.

Assuming that the metabolism and clearance of TEGDMA in humans are similar to those of guinea pigs and mice, it is therefore extremely unlikely that TEGDMA released from restorative materials in humans could have systemic toxic effects.

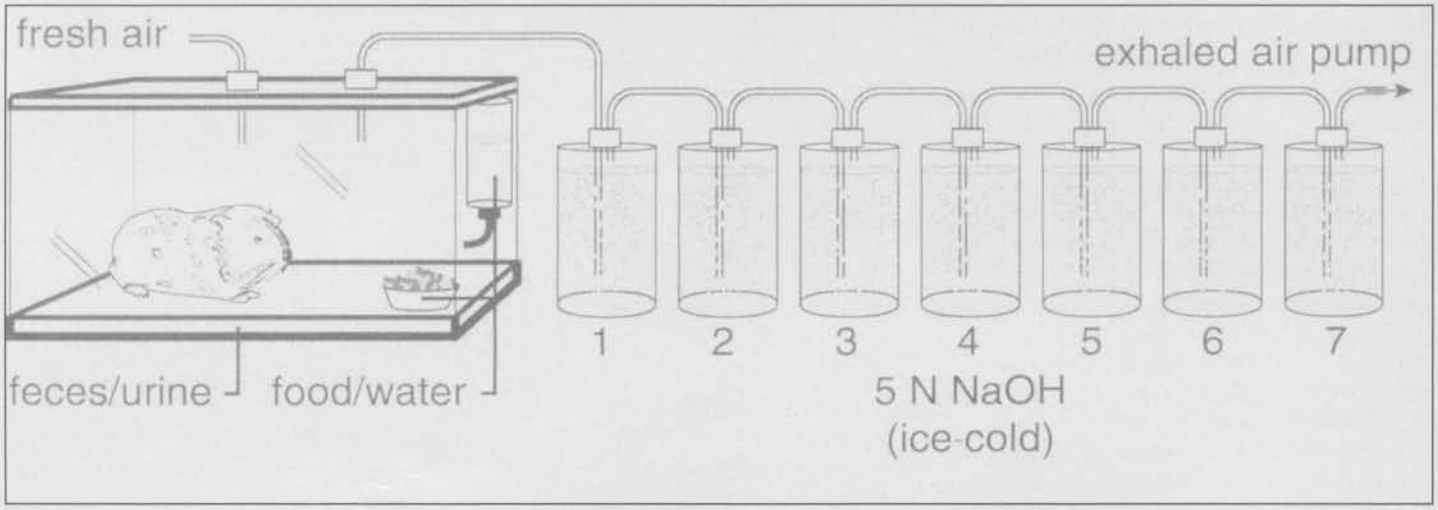

Figure. Metabolic cage with controlled airflow for capturing $^{14}CO_2$. Exhaled carbon dioxide was captured by the pumping of the exhaled air through bottles 1 to 7, each filled with ice-cold 5 N NaOH.

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