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ABSTRACT

Bisphenol-A-glycidylmethacrylate (BisGMA) is used in many resin-based dental materials. It was shown *in vitro* that BisGMA was released into the adjacent biophase from such materials during the first days after placement. In this study, the uptake, distribution, and excretion of [¹⁴C]BisGMA applied *via* gastric and intravenous administration (at dose levels well above those encountered in dental care) were examined *in vivo* in guinea pigs to test the hypothesis that BisGMA reaches cytotoxic levels in mammalian tissues. [¹⁴C]BisGMA was taken up rapidly from the stomach and intestine after gastric administration and was widely distributed in the body following administration by each route. Most [¹⁴C] was excreted within one day as ¹⁴CO₂. The peak equivalent BisGMA levels in guinea pig tissues examined were at least 1000-fold less than known toxic levels. The peak urine level in guinea pigs that received well in excess of the body-weight-adjusted dose expected in humans was also below known toxic levels. The study therefore did not support the hypothesis.

KEY WORDS: BisGMA, metabolism, excretion, organs, guinea pig.

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Distribution and Excretion of BisGMA in Guinea Pigs

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 bisphenol-A-glycidylmethacrylate (BisGMA); and (2) triethyleneglycoldimethacrylate (TEGDMA), which is included to compensate for the high viscosity of the primary resin. Resin composites contain BisGMA in amounts varying from 5-9 wt% (Munksgaard *et al.*, 2000), while fissure sealants contain BisGMA in the range 10-50% (Komurcuoglu *et al.*, 2005).

Direct evidence of BisGMA release from composite resins and fissure sealants into the biophase was provided previously (Tanaka *et al.*, 1991; Spahl *et al.*, 1998; Nalcaci *et al.*, 2006). Released dental material components can be expected to enter the body by 2 different routes after resin placement, *via* the saliva and gastrointestinal tract, plus (if the material is placed on dentin) *via* the dentin and pulp (Hume and Gerzina, 1996).

To test the hypothesis that BisGMA reaches cytotoxic levels in body tissues, and to develop an understanding of the whole-body kinetics of BisGMA, we measured the uptake, distribution, and clearance of [¹⁴C]BisGMA administered by gastric tube and by intravenous injection in guinea pigs.

MATERIALS & METHODS

[¹⁴C]BisGMA was purchased from Perkin Elmer (Boston, MA, USA), dissolved in dichloromethane, and stored at -20°C. Unlabeled BisGMA was obtained from Degussa (Frankfurt, Germany).

In situ Experiments

Guinea pigs were randomized and allotted to 3 groups of 4 animals each. The animals were anesthetized with urethane (1 mg/kg, 4 mL/kg; dissolved in 0.9% NaCl solution). The ductus cysticus was ligated, and a cannula was placed in the bile duct, as described in previous experiments (Reichl *et al.*, 2001). A 1 mL/kg quantity of BisGMA, dissolved in DMSO (final DMSO concentration, 1%), was injected *via* the jugular vein [0.02 μmol/kg body weight (bw), labeled with a tracer dose of [¹⁴C]BisGMA, 0.3 kBq/g bw] (group 1). Control animals (groups 2 and 3) received either 0.9% NaCl solution or DMSO (final DMSO concentration, 1% in 0.9% NaCl solution), each without BisGMA. Blood was withdrawn from the carotid artery. Samples of the bile (0.4 mL) and blood (0.2 mL) were taken every 10 min. The experimental period was 60 min. The organs and their contents were immediately removed and their [¹⁴C]-radioactivity measured.

In vivo Experiments

Adult male guinea pigs (Dunkin-Hartley Pirbright white strain, 400 g), were fed a standard diet and water *ad libitum*. Twelve guinea pigs were allotted to 3 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

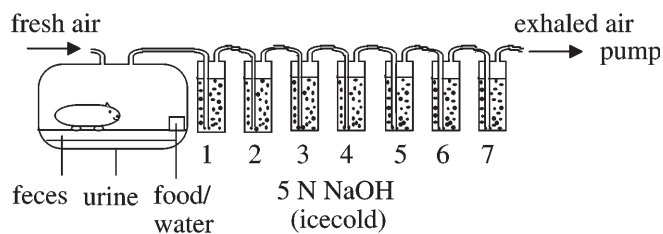


Figure. Metabolic cage with controlled air flow for capturing [^{14}C]-carbon dioxide. Exhaled carbon dioxide was captured by pumping the exhaled air through bottles 1-7, each filled with ice-cold 5 N NaOH.

received BisGMA (0.02 $\mu\text{mol/kg}$ bw, dissolved 1% DMSO solution, labeled with a tracer dose of [^{14}C]BisGMA, 0.3 kBq/g bw) *via* gastric tube (group 4). Control animals (groups 5 and 6) received either 0.9% NaCl solution or DMSO (final DMSO concentration, 1%), each without BisGMA. The volume for the application through the gastric tube was 4 mL/kg. Feces and urine were collected at 1, 2, 4, 6, 8, 12, and 24 hrs after [^{14}C]BisGMA administration, and the [^{14}C]-radioactivity was measured as described below. Twenty-four hrs after the beginning of the experiment, the animals were killed in ether. The organs and their contents were immediately removed and their [^{14}C]-radioactivity measured. Details of organ treatment and the method of determination of radioactivity are in the APPENDIX. Data are presented as means \pm SEM. Statistical significance of the differences between the experimental groups was determined by the Bonferroni-Holm *t* test (Sheskin, 2007).

A second set of 12 guinea pigs was treated as described above, with the addition that each animal was kept in a closed chamber with controlled air flow. The exhaled air was captured during the 24-hour experimental period by flowing through 7 bottles, 1 behind the other, filled with 250 mL ice-cold 5 N NaOH (see Fig.). [^{14}C]-carbon dioxide was captured as [^{14}C]- Na_2CO_3 and the total [^{14}C] activity determined. Urine and feces were collected at 24 hrs after the beginning of the experiment, and the animals were killed and organs taken for analysis as described for the first *in vivo* experimental group.

Declaration

These experiments complied with the current laws of Germany. The animal experiments were approved by the Regierung von Oberbayern, permission no. 211-2531-66/94.

The accession number for [^{14}C] compounds is: 4/4-8816.352-14783, Bayerisches Landesamt für Umweltschutz, Augsburg, Germany.

Guinea Pig Organs

Organs taken 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 , the wash-water was saved, and then the tissues were weighed and homogenized.

Determination of Radioactivity, Guinea Pig Experiments

Tissues were dissolved in tetraethylammoniumhydroxide (TEAH) (20%) in aqueous solution with Omni-Szintisol[®] (both from

Table 1. [^{14}C] Recovery in Guinea Pigs in the Bile and Blood, as well as Summed [^{14}C] Distribution in Organs 1 hr after Intravenous [^{14}C]BisGMA Injection (n = 4; mean \pm SEM)

	[^{14}C] Activity (% of the [^{14}C]BisGMA dose administered) Intravenous Injection	
	Mean	SEM
Bile	12.0	2.1
Blood	1.1	0.1
Organ wash water	0.8	0.2
Σ organs	49.6	5.5

Table 2. [^{14}C] Excretion in Guinea Pigs *via* the Urine, Feces, and Carbon Dioxide as well as Summed [^{14}C] Distribution in Organs 24 hrs after [^{14}C]BisGMA Administration with a Gastric Tube (n = 4; mean \pm SEM)

	[^{14}C] Activity (% of the [^{14}C]BisGMA dose administered) Application with the Gastric Tube	
	Mean	SEM
Urine	7.3	1.1
Feces	5.0	0.8
Exhaled $^{14}\text{CO}_2$	65.3	2.3
Organ wash water	1.2	0.2
Σ organs	5.8	0.9
Total [^{14}C] recovery	84.6	2.8

Merck, Darmstadt, Germany). Radioactivity was determined by the method described by Strugala (1984), with a liquid scintillation counter (2500 TR, Canberra-Packard, Dreieich, Germany).

RESULTS

[^{14}C] Recovery from Guinea Pigs after 1 Hr

During the first hr after [^{14}C]BisGMA intravenous injection, about 12% of the [^{14}C] was excreted *via* the bile, and about 50% remained in the tissues at 1 hr, the time the guinea pigs were killed (Table 1). [^{14}C] activity was rapidly removed from the blood (Table 1). Additional data on [^{14}C] distribution in the bile and blood and [^{14}C] activity in various organs are included in the APPENDIX.

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

During the first 24 hrs after [^{14}C]BisGMA oral administration, guinea pigs exhaled $^{14}\text{CO}_2$ equivalent to about 65% of the [^{14}C]BisGMA dose administered (Table 2). About 7% of the [^{14}C] was excreted *via* urine, and about 6% remained in the tissues at 24 hrs, the time the guinea pigs were killed (Table 2). The total [^{14}C] recovery was about 85% of the [^{14}C]BisGMA dose administered (Table 2). Additional data on fecal and urinary volumes, [^{14}C] excretion, and [^{14}C] activity in various organs are included in the APPENDIX.

DISCUSSION

The administered dose levels were chosen to exceed substantially the body-weight-adjusted dose levels relative to humans for BisGMA released from large composite resin restorations. For the guinea pig experiments, we used the data of Munksgaard *et al.* (2000), who measured extracted quantities from polymerized composites eluted for 7 days with water. The values for various composite components eluted in these chemical experiments ranged from 0.03 to 0.07 wt% in water. The highest eluted BisGMA level into water shown by Munksgaard *et al.* (2000) was from the commercial composite Z100[®] (3M ESPE, Seefeld, Germany), which provided about 1.5 μmol BisGMA from 16 g composite. Assuming the worst case, that all restorations in all teeth are replaced by Z100 (32 teeth; about 0.5 grams *per* tooth/filling = about 16 g Z100), a dose level equivalent to the total amount released from this material was used and adjusted by proportion with the body weight of the average human (1.5 μmol BisGMA/70 kg bw = 0.02 $\mu\text{mol}/\text{kg}$ bw).

Analysis of our data showed that uptake of [¹⁴C]BisGMA from the stomach and intestine of guinea pigs was rapid and essentially complete, and that distribution of [¹⁴C]BisGMA and/or its metabolites throughout the body with these routes of administration was broad. More than 65% of the administered [¹⁴C] was excreted *via* the lungs as ¹⁴CO₂ in guinea pigs, while some 7% of the administered [¹⁴C] appeared in the urine. Most of the [¹⁴C] label was cleared from the body one day following gastric administration.

The cumulative biliary [¹⁴C] excretion was about 2 times higher after 60 min than the cumulative fecal [¹⁴C] excretion after 24 hrs. Therefore, enterohepatic recycling of [¹⁴C]BisGMA and/or [¹⁴C]BisGMA metabolite(s) cannot be excluded. Enterohepatic recycling and similar biological clearances have also been described for HEMA and TEGDMA in guinea pigs (Reichl *et al.*, 2001, 2002). Analysis of the present data indicates the existence of similar mechanisms for uptake and biliary excretion for HEMA, TEGDMA, and BisGMA.

We did not determine where, in the organism, BisGMA was metabolized to carbon dioxide. However, the moderately elevated levels in the lung relative to other tissues after intravenous or oral administration suggest metabolic conversion in that tissue. Furthermore, metabolism of BisGMA and formation of CO₂ from BisGMA appear to be independent of these routes of administration, *i.e.*, metabolism of BisGMA in the stomach and/or in the intestine is very unlikely. Further discussion about possible metabolic pathways and distribution and clearance following intravenous and oral administration is included in the APPENDIX.

Despite the fact that the body-weight-adjusted dose of BisGMA administered was more than 20 times higher than that which would be encountered by a human, the highest levels of BisGMA observed in tissue samples taken from guinea pigs, 1.5 nano M in the lung, 1 hr after intravenous injection are approximately 1000-fold less than the known toxic level for BisGMA (Kostoryz *et al.*, 2003; Schwengberg *et al.*, 2005; Reichl *et al.*, 2006; Schweikl *et al.*, 2006).

Similarly, with a dose well in excess of the weight-adjusted, expected equivalent in humans, the highest [¹⁴C] concentration in the spontaneous urine in guinea pigs was

found 4 hrs after the oral [¹⁴C] application (1.2% in 1.4 mL). Peak levels in blood and kidney tissue occurred much earlier than this, which is consistent with the known time dynamics of urine production and the variable (but expected) delay before urine release. Calculated from this [¹⁴C] peak concentration in the urine at the dose administered, a peak BisGMA concentration in the spontaneous urine can be estimated at about 0.2 nmol/L. This concentration is at least 1/500,000th of the level that decreased the cell survival ratio in kidney cells (Hikage *et al.*, 1999). It is therefore unlikely that BisGMA released from restorative materials could have any systemic, toxic effects. However, it is noted that only general cytotoxic effects were investigated in association with BisGMA, and there may be many as-yet-uninvestigated toxic effects generated in various cells by this substance.

The possibility of allergic responses in patients and in dental workers is discussed in the APPENDIX.

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