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Salivary bisphenol-A levels detected by ELISA after restoration with composite resin

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Bisphenol-A diglycidylether methacrylate (Bis-GMA), which is synthesized from bisphenol-A (BPA), a compound with exogenous endocrine disrupter action, is widely used as a dental material. During clinical filling with sealants and composite resins, the compounds are solidified by polymerization and then used. However, it has been noted that unpolymerized monomers may become dissolved in saliva. In this study using a competitive ELISA system, we investigated the changes in the BPA concentration in saliva after restoration with composite resins. Commercial composite resins from nine companies were tested. Mixed saliva was collected from 21 subjects. Based on the dynamics of salivary BPA detected by this ELISA system, we concluded that several tens to 100 ng/ml of BPA were contained in saliva after filling teeth with composite resin but that sufficient gargling can remove it from the oral cavity. Our data suggest that sufficient gargling after treatment is important for risk management.

1. Introduction

Bisphenol-A (BPA) is a major component of epoxy resin and polycarbonate resin, and it has been suspected in recent years of having endocrine disrupter action as an environmental hormone. It has been reported that 2.3–3.6 ng/ml of BPA is generated in the air [1] and that its influence on the water environment and ecosystem is of concern [2, 3]. Regarding the effects of BPA on animals, vom Saal *et al.* [4] reported that administration of 1/25 of the acceptable daily intake, 2 μg/kg body weight, to pregnant mice induced hypertrophy of the prostate in newborn males, and Takai *et al.* [5] reported that growth of early mouse embryos was promoted.

BPA is widely used as a starting material for sealants and composite resins worldwide. In clinical filling with sealants and composite resins, the compounds are solidified by polymerization and then used. However, it has been noted that unpolymerized monomers may be dissolved in saliva and thus the patient may be exposed to the monomer [6–11]. Recent improvements of instrumental performance have allowed more precise analytical results than those obtained by the previous analytical methods. However, such analyses were performed using liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS), and UV [12], requiring complex pretreatments, and these methods are not suitable for the treatment of a considerable number of samples. Using an enzyme-linked immunosorbent assay (ELISA) system that readily measures serum and plasma BPA without pretreatment by extraction, BPA in saliva samples was measured before and after restoration with various composite resins.

2. Materials

Nine commercially available products were used in this study: A: Z 100 (3 M, St. Paul, MN); B: Progress (Kanebo, Ltd., Tokyo, Japan); C: Palfique Toughwell (Tokuyama Corp., Tokyo, Japan); D: Matafil Flo (Sun Medical Co., Ltd., Shiga, Japan); E: Unifil S (GC Corp., Aichi, Japan); F: Beautifil (Shofu Inc., Kyoto, Japan); G: Xeno CFII (Sankin Kogyo, Tochigi, Japan); H: Prodigy (Kerr Corp., Orange, CA); and I: Cleafil ST (Kuraray Co., Ltd., Okayama, Japan) (Table).

3. Methods

3.1. Method of restoration with composite resin

After informed consent was obtained, 21 patients underwent cavity preparation. All of the method in this

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study followed "The Guidelines for Human Studies". Almost the same size of cavity was used in each treatment. The bonding agent provided by each maker was applied to a prepared cavity, which was then irradiated with visible radiation for 30 s. according to the manufacturer's recommended procedure. Each cavity was then filled with 0.1 g of composite resin, irradiated with visible radiation for one min, and ground with a silicon point for polishing.

3.2. Collection of saliva samples

Saliva was collected for five minutes while the subject bit a paraffin pellet used in saliva sampling for oral bacterial testing. Each specimen was centrifuged at 3,000 rpm for 10 min, and the supernatant was used. Samples were collected before filling, immediately after filling with composite resin, and after gargling with tepid water at about 37 °C for 30 s. All samples were analyzed after incubation at 4 °C for 24 h in glass tubes. Aloka RIA Program ARCAS (Aloka Co., Ltd., Tokyo, Japan) was used for data management. In one patient treated with composite resin-A (A-4), BPA in saliva was measured over 120 h after gargling as described above.

3.3. Measurement of BPA

BPA was measured using the BPA ELISA 'EIKEN' Kit (Eiken Chemical Co. Ltd., Tokyo, Japan). This method is a competitive enzyme linked immunosorbent assay (ELISA) developed by Ohkuma *et al.* [13] that measures BPA in biological specimens such as serum and plasma. To the secondary antibody-coated microplates, 20 μl of the standard BPA or sample, 50 μl of enzyme-labeled antigen [horseradish peroxidase labeled BPA-4-carboxyphenolether (CPhE)], and 50 μl of anti-BPA serum were added and reacted at room temperature for one hour. After the reaction solution was removed, 300 μl of washing solution was added to each well and removed. After this procedure was repeated three times, 100 μl of o-phenylenediamine (OPD) solution was added. After the enzyme reaction proceeded at room temperature for 30 min, the reaction was stopped by adding 100 μl of 2 N sulfuric acid, and the optical density (OD) was measured at 492 nm.

4. Results

The composite resins used in this study are summarized in the Table I. The composite resins in A, C, F, G, H, and I are Bis-GMA monomer-based composite resins and bonding agents, and those in B, D, and E are urethane dimethacrylate (UDMA) monomer-based composite resins and bonding agents. In the four patients treated with composite resin-A, the salivary BPA levels before and after restoration and after gargling were 0.3–2.0 ng/ml (mean ± standard deviation: 0.87 ± 0.69 ng/ml), 21.0–60.1 ng/ml (32.1 ± 16.27 ng/ml), and 1.6–4.7 ng/ml (3.1 ± 1.47 ng/ml), respectively (Fig. 1). In one (A-4) of the above patients, saliva was collected for five days after gargling by the same procedure, and BPA was measured. The BPA level varied within the range from that before restoration to that after gargling within a half day; then it converged to the level before restoration (Fig. 2). When we tested C, F, G, H and I, which are made of the same Bis-GMA as A, the BPA level was low even immediately after restoration in those patients treated with G and I. In contrast, in three patients treated with F, the BPA level tended to be high, and a higher level was also present even after gargling (Fig. 1). In patients treated with the non-Bis-GMA materials, the BPA concentration was 40 ng/ml or lower, even in the patient with the highest level immediately after restoration (D-1); these data indicate that the level was generally low (Fig. 3).

TABLE I Composite resins and bonding agents used in this study

	Restoratives (Manufacturers)	Source monomer	Bonding agent
A	Z 100 (3 M)	Bis-GMA/TEGDMA	Bis-GMA
B	Progress (Kanebo)	UDMA/TEGDMA	UDMA/TEGDMA
C	Palfique Toughwell (Tokuyama)	Bis-GMA	Bis-GMA
D	Metafil Flo (Sun Medical)	UDMA/TEGDMA	UDMA
E	Unifil S (GC)	UDMA	UDMA
F	Beautifil (Shofu)	Bis-GMA/TEGDMA	Bis-GMA
G	Xeno CFII (Sankin Kogyo)	Bis-GMA	Bis-GMA
H	Prodigy (Kerr)	Bis-GMA/TEGDMA	Bis-GMA
I	Cleafil ST (Kuraray)	Bis-GMA/TEGDMA	Bis-GMA

Bis-GMA: Bisphenol-A diglycidylether methacrylate.
TEGDMA: Triethylen glycol dimethacrylate.
UDMA: Urethane dimethacrylate.

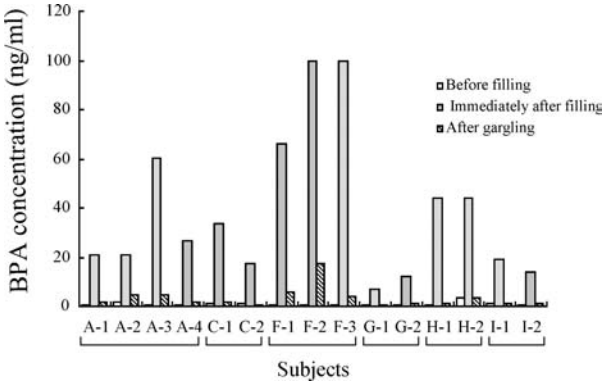

Figure 1 Salivary BPA concentrations before and after restoration with Bis-GMA- and TEGDMA-based composite resins.

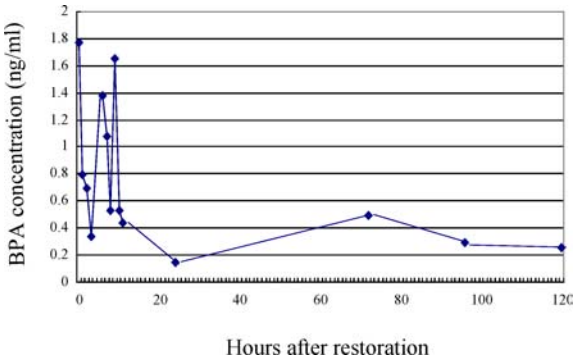

Figure 2 Time course of BPA concentration after restoration with composite resin-A, which is mainly composed of Bis-GMA.

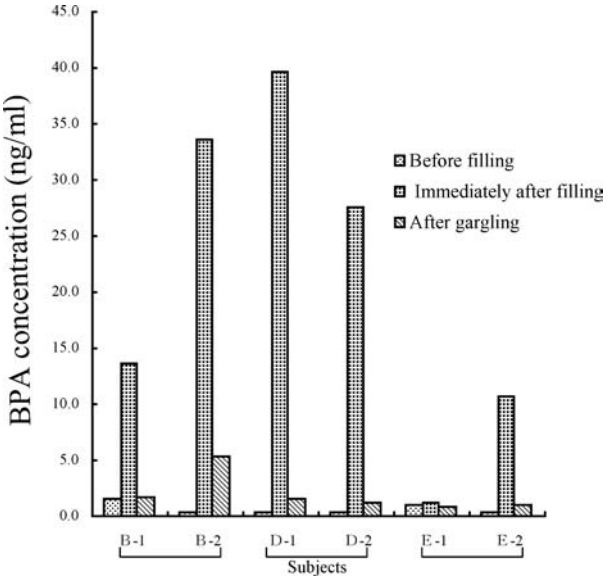

Figure 3 Salivary BPA concentrations after restoration with urethane monomer-based composite resin

5. Discussion

Olea *et al.* [6] initially reported the problem of dissolution of BPA. The amount of BPA dissolution from one resin-based sealant used in their study was abnormally high, and this product contained Bis-DMA. Many researchers have performed additional studies [7, 8, 14–17]. Recently, Tarumi *et al.* [18] analyzed the BPA content in three types of sealant and five types of bonding material by HPLC equipped with a UV detector and evaluated the estrogen activity in these materials by a receptor gene assay using Hela cells. They tested three types of sealant but did not detect BPA in any of the products. However, estrogen activity was detected in two products containing Bis-DMA, and they concluded that this activity was due to Bis-DMA, not due to BPA. Ohsaki and Imai [19] performed tissue analysis of commercial Bis-GMA, and confirmed that in addition to Bis-GMA and its structural isomer, iso-Bis-GMA, 2,2 [4-(2-hydroxy-3-metacryloiroxy-1-propoxy)-4-(2,3-dihydroxy-1-propoxy)] diphenylpropane (Bis-GMA-H), which possesses a structure in which one metacrylate ester bond of Bis-GMA is hydrolyzed, was present. They pointed that this Bis-GMA-H may appear as a peak overlapping the BPA peak under certain analytical HPLC conditions. The high BPA content reported by Olea *et al.* [6] might be due to inappropriate separation conditions.

Because BPA may dissolve from the inner coating materials of canned food, the subjects were instructed to avoid drinking canned beverages from one week before their dental treatment in our study. As in the study reported by Noda *et al.* [12], dissolution of BPA from non-Bis-GMA composite resins was detected in this study. Dissolution of BPA from non-Bis-GMA suggests slight contamination during the synthesis process. However, the level after gargling was very low. The amount of BPA in saliva collected before treatment was equal to that in the umbilical cord, and this detection in saliva suggested contamination *via* pathways other than dental treatment.

Not only Bis-GMA but also many BPA analogues are used as monomers in sealants, composite resins, and bonding agents for dental treatment [19]. Based on the published range, these dental materials are not composed of a single compound, and removal of simple unpolymerized compounds, byproducts, and impurities is difficult at the time of polymerization from a practical standpoint. This fact makes any discussion of safety confirmation of materials concerned in endocrine disrupter action complex. Is BPA released? What level is the detection level? It is undeniable that separation analysis by HPLC and GC/MS is too complex for frequent analysis near clinical practice [12, 14, 15, 17, 20, 21]. Although the values measured using this ELISA system may include a large amount of crossed compounds among impurities and polymerization byproducts contained in dental materials composed of multiple ingredients, because the cross-reactivity among Bis-DMA, TEGDMA, and HEMA contained in monomers is low, only salivary BPA may have been detected [13]. Based on the dynamics of salivary BPA detected by this ELISA system, we conclude that several tens to 100 ng/ml BPA were present in saliva after filling cavities with composite resin but that sufficient gargling can remove this compound from the oral cavity. After removal by gargling, the BPA concentration converged to a constant level after half a day. Depending on the restorative material, the concentration can be reduced to a level lower than 10 ng/ml even immediately after restoration. Therefore, for dental treatment of pregnant women and children, who are readily affected by endocrine disrupters, it is important to insist upon sufficient gargling after treatment and/or to select materials with consideration of risk management.

It has been reported that BPA is degraded slowly by gram-negative aerobic rods [22], and possible conversion to stilbene during the bacterial degradation process is being clarified. The questions of how oral bacteria degrade BPA dissolved in saliva, how they convert BPA to stilbene, and which species of bacteria are involved in this process remain for future clarification.

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