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Toxicological Aspect of Orthodontic Bonding Materials

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To cite this article:

A. K. Rai, A. Ansari, A. Gera, A. K. Jain
Toxicological Aspect of Orthodontic Bonding Materials
Virtual Journal of Orthodontics
[serial online] 2011 November, 9 (3)
Available at: <http://www.vjo.it>

Virtual Journal of Orthodontics

Dir. Resp. Dr. **Gabriele Floria**

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CCIAA n° 31515/98 - © 1996
ISSN-1128-6547 NLM U. ID:
100963616 OCoLC: 40578647

Abstract:

Composites are the most commonly used materials in the contemporary orthodontic practice for bonding attachments to the tooth surface. Superior aesthetics, bond strength and adequate working time are the most common advantages cited. The literature however is scarce about the potential adverse effect of these materials. The goal of this article is to provide current information about the potential toxic effects of composite adhesive with primary focus on its estrogenicity. Three topics primarily discussed in this article are, the development of BIS-GMA, the effect of estrogenic hormones and its analogue and the potential toxic effect of BIS-GMA. Finally, some clinical recommendations are provided to minimize the exposure to this chemical from dental composite.

Keywords: Composites, BIS-GMA, bonding material, estrogenicity, toxicity.

Introduction

As early as 1936, Dodds and Lawson¹ reported the estrogenicity of some diphenyl compounds containing two hydroxyl groups in para positions. One such derivative, bearing two methyl groups is known as bisphenol A (BPA). In 1996², investigators in Spain and Tufts University reported levels of BPA to be between 3.3-30.0 micro gm/ml saliva sample collected one hour after DeltonTM sealant was placed. BPA is a xenoestrogen that can bind to the esterophiles stimulating their growth²⁻⁶. The acute and chronic, local and systemic action of BPA has off-lately been a bone of contention in the dental quarters.

To understand the potential risks associated with BIS-GMA, we must understand both the chemistry, including synthesis of BIS-GMA, and the biological interaction between the bisphenol A molecule (used during the synthesis of BIS-GMA) and estrogen receptors.

Development of BIS-GMA

During World War II, German researchers developed a chemical process that could be used to cure dental methacrylates at room temperature⁷. To reduce polymerization shrinkage, researchers added inert filler particles to the selfcuring methacrylate resin. Bowen attached methyl methacrylate groups to the terminal end of the epoxy resin to overcome its moisture sensitivity and developed a new resin called bisphenol A glycidyl methacrylate, or BIS-GMA⁸. To decrease its viscosity different monomers with lower viscosities as triethyleneglycol dimethacrylate, or TEGDMA were added.

Bowen, in 1965, described three main ways to synthesize BIS-GMA⁷. The first, attaching methacrylate groups to hydroxy glyceryl groups, which, in turn, were linked to phenoxy groups to form BIS-GMA. The second synthetic method was to condense the sodium salt of bisphenol A with an equivalent amount of the reaction product of glycidyl methacrylate and anhydrous hydrochloric acid. The third, which Bowen preferred when he submitted his patent application, was to combine two moles of glycidyl methacrylate with one mole of bisphenol A. A tertiary amine was added to catalyze the addition of the phenolic hydroxyl groups to the epoxide groups.

From a biological point of view, several problems exist with the above syntheses of BIS-GMA monomer. The first method can produce residuals of diglycidyl ether of a bisphenol in dental composites and cause allergic reactions⁹. The second method results in residues that can induce estrogenic effects² (for example, the salt of bisphenol A) or produce allergic reactions (for example, glycidyl methacrylate). Finally, the third method could leave both glycidyl methacrylate and bisphenol A as impurities, causing allergic and estrogenic effects, respectively, from poorly purified BISGMA resins.

The estrogen family and its action

Estrone, estradiol and estriol are the naturally occurring estrogen molecules found in humans. Estradiol is the most potent estrogen and can be converted metabolically to estrone or estriol. Structure-activity relationship of Estrogen receptors for estrogens have shown selective, high-affinity binding of steroidal and nonsteroidal compounds that contain the

phenolic A ring of the cyclopentanoperhydrophenanthrene structure. Hence, agents containing a phenolic ring, such as diethylstilbestrol or bisphenol A, have the ability to activate estrophiles.

Effect of estrogen and its analogue Estradiol is the most abundant estrogen found in premenopausal women, while estrone is the most abundant estrogen in postmenopausal women and in men^{10,11}. The principal biological activities of estrogens in women include development, growth and maintenance of secondary sex characteristics; stimulation of uterine growth; control of the pulsatile release of luteinizing hormone from the central nervous system; thickening of the vaginal mucosa; and ductal development in the breast. In men, the physiological significance of estrogens is largely unknown, but they may be involved in the regulation of androgen and estrogen levels as well as sexual behaviour¹².

Evidence suggests that stomatic tissues in the mouth are modulated by estrogens¹³. For example, during pregnancy, the prevalence and severity of gingivitis has been reported to be elevated leading to greater gingival probing depths^{14,15} increased bleeding on probing or toothbrushing¹⁵ localized gingival enlargement and elevated gingival crevicular fluid production¹⁶.

Toxicity assessment of BIS-GMA

The available data is based on the research involving evaluation of the effects of agents on cells in culture or in cells found at the site of action in the body.

Cell culture experiments

BISGMA-based resins contain many chemicals, including BISGMA and minor amounts of impurities such as bisphenol A and/or diglycidyl ether of bisphenol A. Other monomers, such as TEGDMA, BIS-DMA and bismethacryloyloxyethoxyphenylpropane, are also added to the BIS-GMA monomer to change the rheology of the resin phase.

Olea's² study was the first to assess the estrogenic effects of dental resins and found that saliva samples collected one hour after sealants were placed (approximately 50 milligrams of sealant per subject) contained variable amounts of bisphenol A (ranging from 3.3 to 30 µg/ml). They concluded that BIS-GMA, by itself, was unable to stimulate proliferation of breast cancer cells in culture. In contrast, bisphenol A was shown to be an estrogenic compound capable of stimulating the number of cells and the progesterone receptor content of breast cancer cells, but at 2,500 times the concentration necessary for estradiol to produce similar effects¹⁷. However, this study evaluated only one cell line (obtained from a pleural effusion derived from a human breast adenocarcinoma) and a small number of parameters.

Hashimoto et. al.¹⁸, studied the estrogenic activities of 10 chemicals [bisphenol-A (BPA), bis-2-hydroxypropyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA), dibutyl phthalate (DBP), n-butyl benzyl phthalate (BBP), n-butyl phthalyl n-butyl glycolate (BPBG), di-2-ethylhexyl phthalate (DEHP), and di-2-ethylhexyl adipate (DOA)] by a reporter gene assay (yeast two-hybrid system) and an estrogen/estrogen receptor

(ER-α) competition binding assay (fluorescence polarization system). The result of this study showed that BPA and BBP had estrogenic activity at the concentration tested. The estrogenic activity in this assay was comparable to that reported by Villalobos et.al.¹⁹.

However as Villalobos et al.¹⁹ pointed out that Olea used E-screen assay which was based on the ability of MCF-7 cells to proliferate in the presence of estrogens, differences in sensitivity to estrogen between MCF-7 and other cells could lead to different results. Although the estrogenicity of BIS-GMA-based dental resins is still not a well established fact, in vitro experiments have identified components that are released from such resins. Substances released from orthodontic composites may cause a reaction (inflammation or necrosis) in adjacent tissues, such as the oral mucosa and gingiva, or alveolar bone. There are several ways that materials may influence the health of soft tissues-by delivering water-soluble components into the saliva and the oral cavity as well as by interacting directly with adjacent tissues. In orthodontic treatments, controlling periodontal tissue health is important. It is hypothesized that the orthodontic adhesives can induce gingival inflammation. The prostaglandin E2 inflammatory mediator is known to exert diverse physiologic actions in different tissues and to be involved in the inflammation process^{20,21}. The enzymes, including phospholipase A2 and cyclooxygenase (COX), regulate the production of the prostaglandin. Prostaglandins are produced by the action of COX enzymes on the free arachidonic acid liberated from membrane phospholipids by phospholipases. Prostaglandin endoperoxide H synthase (also

referred to as COX) is the rate-limiting enzyme for the production of prostaglandins and thromboxanes from free arachidonic acid²². Two forms of COX have now been described: a constitutive enzyme (COX-1), present in most cells and tissues and an inducible isoenzyme (COX-2) expressed in response to cytokine growth factor, lipopolysaccharide, and other stimuli²³. COX-2 is an intermediate response gene that encodes a Mr71000 cytoplasmic protein that is up-regulated at sites of inflammation²⁴. COX-2 is constitutively expressed in the brain, kidney, and testes; however, in most other tissues its expression is induced by pro-inflammatory or mitogenic agents, including cytokines, tumor promoters, endotoxins, and mitogens.

Various studies have been conducted exploring the cytotoxic profile of these composite on the local tissue analogue models.

Huang et.al.²⁵ evaluated the in vitro inflammation behaviour of the resin base and resin modified glass ionomer base adhesives after contacting primary human gingival fibroblasts and concluded that all orthodontic adhesives induced COX-2 protein expression in human gingival fibroblasts. The exposure of quiescent human gingival fibroblasts to adhesives resulted in the induction of COX-2 mRNA expression. For orthodontic patients with gingival inflammation, except for those with oral hygiene problems, the activation of COX-2 expression by orthodontic adhesive may be one of the potential mechanisms.

Malkoc et.al.²⁶ evaluated the cytotoxic effects of five different light-cured orthodontic composite namely Heliosit Orthodontic (Ivoclar), Transbond XT (3M Unitek), Bisco

ORTHO (Bisco), Light Bond (Reliance), and Quick Cure (Reliance) composites on viability and cellular morphology of permanent mouse fibroblast (L929) cells. L929 fibroblasts and gingival fibroblasts have been shown to have similar cytotoxicity levels. Consequently, L929 fibroblasts make a useful screening model for in-vitro toxicity testing of dental materials. The result of this study showed that Transbond XT was significantly cytotoxicity compared with the control group.

Hansel et.al.²⁷ investigated the influence of base monomers (bis-GMA, UDMA) and co-monomers (TEGDMA, EGDMA) on the in vitro proliferation of caries-relevant bacteria. They found that the base monomers had no influence or only a slightly growth inhibiting effect on these cultures, but that both of the co-monomers tested (TEGDMA, EGDMA) promoted bacterial proliferation. Because these substances usually leach from resin-based composites at higher concentrations than base monomers do, an overall increased bacterial growth may be the consequence in the presence of resin-based composites. Hanks et.al.²⁸ showed that Bis-GMA concentrations of 5 micro mol/L produced a depression of DNA synthesis in mammalian fibroblast.

Yoshii et al.²⁹ examined the relationship between the structure and cytotoxicity of monomers used in dental resin materials, and reported that the cytotoxicity ranking of monomers was Bis-GMA>TEGDMA>HEMA>MMA.

Eliades et.al.³⁰, 1995 showed that there was a statistically significant linear correlation between the DC% of orthodontic adhesives and the residual Bis-GMA concentrations.

Jagdish et.al.³¹ correlated degree of conversion of five orthodontic adhesive to their cytotoxicity and reported that Single-cured systems are superior to dual-cured systems in exhibiting comparatively less toxicity and higher DC. A significant positive correlation however was not established between cytotoxicity and DC.

In situ experiments

Sohoel et al.³² tested two different BIS-GMA/TEGDMA containing resins that were used for the bonding of brackets. Both substances generated a sensitization in 50% of the experimental animals, with a subsequent allergic reaction. Using a murine model, Mariotti et.al.³³ investigated the physiological and biochemical effects of commercially used BIS-GMA to determine if estrogen-sensitive reproductive tissues, such as the uterus, could be stimulated to grow. Their experiments showed these BISGMA solutions to be marginally estrogenic in the uterus. In this study BIS-GMA injected subcutaneously was at a concentration far higher than those monitored in saliva were unable to stimulate increases in the cell number or cell size of reproductive organs in mice, but were able to stimulate modest increases in the weight and collagen content of the uterus.

Toxicological studies in laboratory animals have shown a wide range of estrogen-response mechanism-mediated effects after low-level in utero BPA exposures (20-400 µgm/kg/ day)³⁴. In males, low-dose BPA exposures of rodent fetuses produced postnatal estrogenic effects, including decreased sperm production³⁵ and increased prostate weight³⁶; in females, it caused disruption of sexual differentiation in the brain³⁷, alteration in mammary gland

development³⁸, altered vaginal morphology³⁹, accelerated growth and puberty⁴⁰, and alterations in estrous cyclicity⁴¹. Furthermore, low-dose BPA exposures disrupted meiosis in rats, leading to aneuploidy⁴², the chromosomal abnormality in humans most commonly identified as resulting in pregnancy miscarriage, or, if the pregnancy is taken to term, mental retardation in offspring⁴³. BPA also has been shown to be a thyroid hormone receptor (THR) antagonist that disrupts THR-mediated transcription in rodents⁴⁴. In humans, BPA concentrations have been associated with both polycystic ovary disease and obesity in women⁴⁵ and the disruption of secretion of gonadotrophic hormones in men.

Davidson⁴⁶ used Hamster model to test the tissue response of skin, oral mucosa and gingival to six adhesives and found that no consistent inflammatory pattern was seen in all but one product Right on TM which caused gross irritation in three to four days of application. Al-Hiyasat⁴⁷ demonstrated the adverse effect of leached substances from dental composites on the fertility of male mice and showed that the testicular sperm count and daily sperm production of the males in the test group relative weights of the testes and seminal vesicles were significantly reduced.

Tell et.al.⁴⁸ examined the potential toxic effects of several orthodontic adhesives (Monolok [Rocky Mountain/Orthodontics, Denver, Colo.]), Unite [Unitek Corporation, Monrovia, Calif.], One to One [TP Laboratories Inc., La Porte, Ind.], Adaptic [Johnson & Johnson, New Brunswick, N.J.], Orthomite [Rocky Mountain/Orthodontics]) immediately after polymerization and at various time intervals up to 2 years after

polymerization. They found that all materials tested showed cytotoxic effects immediately after polymerization and that the toxic effect decreased with time and after polymerization. However, even 2 years after the initial polymerization, toxicity was still evident in all adhesives but Orthomite.

In many cases, the risk of the adverse effects of biomaterials is much higher for the dental team than for the patients because of chronic exposure of the dental team and manipulation of the materials when they are being placed, set or removed. Nathanson et.al.⁴⁹ described a patient with delayed hypersensitivity to dental composite material. The response included itching on hands and face and spots turning into blisters on the face and mild respiratory difficulty. It is thus the responsibility of the orthodontist to inform the affected personnel that the dental materials used by orthodontists can pose some risk to the patient and the dental team.

Pharmacokinetics and metabolism

BPA is weakly estrogenic in in-vitro screening assays. However, because of its low protein binding affinity, more unbound BPA may be available in vivo, potentially rendering it more estrogenic than observed in laboratory studies⁵⁰.

Atkinson et.al.⁵¹ state that BIS-DMA is converted rapidly into BPA in presence of salivary enzymes and could account for the finding of BPA in clinical samples collected after the placement of certain dental composites. In studies by Hamid⁵² and Müller⁵³, similar resins were stored in distilled water or artificial saliva, and TEGDMA was the main substance detected in the storage media. These findings show that the composition of the medium (for example,

saliva) surrounding a restoration will have a significant impact on the amount of leachable components of the resin.

Joskow et.al.⁵⁴ reported salivary and urinary concentrations of BPA in 14 dental patients who received two different brands of dental sealants namely Helioclear F (Ivoclar Vivadent, Amherst, N.Y.) or Delton Light Cure (LC) Opaque (Dentsply/Ash, Dentsply International, York, Pa.). BPA leaches from Delton LC, a sealant without the ADA Seal of Acceptance, but negligible amounts leach from Helioclear F, which carries the ADA Seal of Acceptance. After Delton LC placement, saliva BPA concentrations increased dramatically. Furthermore, urinary BPA concentrations remained elevated for at least 24 hours after placement. Crude dose estimations show that acute BPA doses from Delton LC placement may result in low-dose exposures that are within the range at which estrogen receptor-mediated effects are seen in rodents.

Climie and colleagues⁵⁵ showed that 80 percent of a single oral dose of glycidyl ether of bisphenol A was eliminated in the feces and 11 percent in the urine zero to three days after administration in mice. These findings suggest that most of glycidyl ether of bisphenol A was not easily absorbed from the GI tract, but it is unclear if BIS-GMA and BIS-DMA (BISDMA being another bisphenol A-based resin present in some sealants and composites) would be equally difficult to absorb.

Little is known about BIS-GMA metabolism in the human body enzymes, such as esterases, attack dental composite resins. These proteins catalyze the hydrolyses of ester linkages. Munksgaard et.al.⁵⁶ incubated

a variety of dental resins (diethyleneglycol dimethacrylate, urethane dimethacrylate, TEGDMA, decylmethacrylate, laurylmethacrylate, BIS-GMA and 2-hydroxypropylmethacrylate) with porcine liver esterase. They found that methacrylic acid, or MAA, was released as a by-product, suggesting that the resins were hydrolyzed. From these studies, one can conclude that a hydrolase (esterase) can catalyze the hydrolysis of monomethacrylates and dimethacrylates in the polymerized and unpolymerized state, and that the hydrolytic process would increase the hydrophilicity of the resin surface.

Nathanson et al.⁵⁷ tested seven commercially available sealants namely Delton, Concise, Heliobond, Prisma: Shield, Seal-Rite (BIS-GMA), Seal-Rite (UDMA) and Defender and reported that no BPA was detected in elute from any of the sealants tested.

An attack of the ester linkages in BIS-DMA could result in the formation of bisphenol A, but a similar attack on BIS-GMA will not result in the formation of bisphenol A. Thus, some of the findings presented by Olea² may be related to the presence of BIS DMA and not BIS GMA.

Clinical recommendations

To reduce the potential cytotoxic effects, several precautionary measures can be followed. The clinician should use minimal material as needed, with care to remove excess adhesives, particularly in areas where it may come in contact with the sub-gingival and inter-proximal tissues.

Various clinical techniques are available for removing the layer of unreacted components

to reduce patient's exposure to them. Some practitioners prefer not to remove this layer and instead merely warn patients that they can expect to experience an unpleasant taste for a short time. Rueggeberg⁵⁸ evaluated the efficacy six surface treatment methods to remove oxygen inhibition layer and thereby minimize patients exposure to uncured composite. The test method ranged from no treatment (the control) to a 20-second exposure to an air/water syringe spray, 20 seconds' manual application of a wet or dry cotton roll, 20 seconds' manual application of pumice with a cotton pellet, and 20 seconds application of water and pumice slurry using a prophyl cup on a slow-speed hand-piece. This study showed that pumice slurry is significantly more effective in removing the oxygen inhibited layer (from 93 percent to 95 percent of the untreated control values) from freshly cured sealants than any of the methods evaluated. When sealants are applied, they should be painted conservatively and localized to the tooth surface where the bracket is to be placed, avoiding gingival contact wherever possible.

Shinya⁵⁹ proposes that the degree of monomer conversion of the light-curing adhesive resin under stainless steel bracket can be improved by adding a thin layer of glassfiber-reinforced composite between the bracket and adhesive resin.

Conclusion

Dental monomers available in the market do fulfill Environmental Protection Agency standards regarding maximal bisphenol A content; nonetheless, these standards are based on toxic effects of bisphenol A rather than on their estrogenic effects. The American Dental Association (ADA) maintains that the

products carrying its Seal of Acceptance do not release detectable (> 5 ngm/ml.) of BPA. It is likely that the estrogenic effect that might be induced from a newly placed resin will decrease over time. However, this does preclude the possibility of some additive or synergistic effect with other xeroestrogens present in the mouth. Based on existing research, we must accept that certain impurities may be present in some BIS-GMA-based resins, and these impurities, when released are potentially estrogenic. However, the amounts of bisphenol A that may be present as an impurity or produced as a degradation product from dental composite is quite small and possibly far below the doses needed to affect the human reproductive tract. However, long term studies to prove the same are warranted until then caution is the best discretion.

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