Effects of Bisphenol A on the Dental Pulp

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Michel Goldberg

13.1 Introduction

The xenoestrogen bisphenol A (BPA) is widely used in various food and packaging consumer products, in the manufacture of polycarbonate plastics and epoxy resins. It is released from food and beverage containers, baby's bottles, children's toys, and dental restorative materials, including occlusal sealants supposed to prevent the development of carious decays. It produces numerous adverse endocrine and developmental effects in rodents, resulting in general cytotoxic and pathologic outcomes. Some local effects are closely associated with this family of endocrine-disrupting compounds. What is induced is related to the estrogenic properties of BPA and resulting from alterations of synthesis of estradiol and testosterone. These effects are interfering with receptor binding. Irregular cycles, multiple ovarian cysts, reduction in primary follicles, neonatal mortality, sexual dysfunctions, and decreased libido have been reported as undesirable or adverse effects. Epigenetic effects are associated with an increased risk of cancer, namely, breast and prostate malignancies. Low-dose BPA exposure seems to increase adipogenesis in female animals, obesity, non-insulin-dependent diabetes mellitus, allergies, asthma, autism, cognitive decline, memory impairment, depression, and anxiety [1]. In addition to these well-documented general effects, many questions are related to the risks due to release of BPA after the dental restorations after a carious lesion or after the sealing of pits and fissures.

13.2 Cytotoxic Effects and Induced General Pathologies

The lower dose inducing cell damage is determined by the *no observed adverse effect level* (*NOAEL*). It was evaluated by the Food and Drug Administration to be as low as 5 mg BPA/kg body weight (bw)/day. However, according to safety authorities and protection agencies, the *tolerable daily intake* (*TDI*) considered as a reference would be a dose of 0.05 mg/kg bw/day. The issue of the dose is still a matter of debate, but it is clear that doses below the NOAEL have significant effects. According to Moon et al. [2] doses of BPA below the NOAEL induce mitochondrial dysfunctions in the liver and are associated with an increase in oxidative stress and inflammation.

Low concentrations of BPA induce lipid accumulation in hepatic cells, mediated by the production of reactive oxygen species in the mitochondria of HepG2 cells. Mitochondrial dysfunctions,

M. Goldberg, DDS, PhD
Department of Oral Biology,
Institut National de la Santé et de la Recherche Médicale, Université Paris Descartes,
45 Rue des Saints Pères, Paris 75006, France
e-mail: mgoldod@gmail.com,
michel.goldberg@parisdescartes.fr

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including ROS production, lipoperoxidation, and the release of proinflammatory cytokines, are contributing to steatosis. They result from low concentration of BPA [3].

Traditional classical dogma in toxicology was "the dose makes the poison." Evolution of the concept suggests that effects may be detected with low doses below that used for traditional toxicological studies. In addition non-monotonic dose-response should also be taken into account. The effects of low doses cannot predict the effects observed at higher doses [4]. This implies that the effects of low doses have to be taken into consideration in terms of undesirable effects and of possible induced pathologies.

Cabaton et al. [5] have reported the effects of low doses of bisphenol A on the metabolome of perinatally exposed CD-1 mice (a method used for determining the metabolic changes to nutritional, pharmacological, and toxic stimuli). Dose-dependent variations in glucose, pyruvate, some amino acids, and neurotransmitters were identified, supporting that low dose of the endocrine disruptor BPA administered from day 1 up to day 21 interferes and disrupts the global metabolism.

13.2.1 Determination of Blood, Urine, Saliva, and Sweat Parameters of Excretion

- Normal values and concentrations found after BPA treatment were reported in body fluids.
- *Blood*: No BPA was found in blood samples prior or after dental treatment.
- *Saliva*: Olea et al. [6] collected the saliva 1 h before the application of cured sealants. After treatment, all saliva samples contained BPA in amount ranging from 90 to 931 μg. In control patients, BPA was detected in the saliva of all patients prior to the placement of the sealants and ranged between 0.07 and 6.00 ng/ml at baseline. Three hours after treatment, the salivary concentration peaked and returned to the baseline level within 24 h. Low peak levels were 3.98 ng/ml (one sealant application alone), whereas 9.08 ng/ml in the high-dose group (more than four sealants) [7]. Altogether, the different clinical studies available conclude that the highest level of BPA reported in saliva from dental sealants is more than 50,000 lower than the lethal dose 50 (LD50) values reported for BPA. This allows some researchers to conclude that human exposure to BPA from dental resins is minimal and poses no known health risk [8]. This contradicts some findings establishing that some low-dose effects of BPA are at the origin of undesirable effects.
- The daily *urinary* BPA excretion gave a median value of 1.2 μg/day, far below the tolerable daily intake recommended by the European Commission in 2002 [9].
- *Human excretion of sweat*: Monitoring the bioaccumulation of BPA in blood, urine, and sweat, Genuis et al. [1] concluded that blood and urine testing might underestimate the total body burden of the potential toxicant. By contrast, they considered that sweat analysis should be considered as an additional method to follow accurately the accumulation of BPA and its elimination.

Therefore, metrologic evaluations should keep attention on the false body fluids and not on what should be considered as significant.

13.2.2 Toxicogenomics and Adverse Health Effects

BPA exhibits toxicogenomics and undesirable effects on human health owning to the 89 common interacting genes/proteins. These genes/proteins may serve as biomarkers to assay the toxicities of the different chemicals leached out from the widely used plastics.

Bisphenol A acting as an endocrine disruptor is implicated in the feminization in various organs and displays various estrogenic effects. Due to a competitive ligand binding, BPA is bound to estrogen receptors α and β. The density of mammary buds was increased in BPA-exposed monkeys, leading to precancerous forms [10].

13.2.3 Precancerous and Cancerous Effects

Exposure to low doses of BPA resulted in significant alterations in gland morphology, which varied to subtle effects on mammary gland development when the exposure period occurs in adulthood, leading from precancerous to cancerous lesions. Prenatal exposure to relevant doses of BPA increases the number of intraductal hyperplasia and ductal carcinoma. Acevedo et al. [11] reported that the environmental levels of BPA during gestation and lactation induce mammary gland neoplasms even in the absence of any additional carcinogenic treatment.

13.2.4 Other Adverse Effects

13.2.4.1 Brain Development

- Prenatal and lactational exposures to low doses of BPA show effects on brain development in mice.
- In the adult mice brains, abnormal neocortical architecture and abnormal corticothalamic projections persisted in the group exposed to the BPA. Epigenetic alterations might trigger some of the effects on brain development after exposure to BPA [12].
- High-dose BPA impairs hippocampal neurogenesis in female mice across generations. This shed lights on another important feature: the *transgenerational effect*. The evaluation of transgenerational effects of BPA on hippocampal neurogenesis showed that when pregnant female mice were exposed to BPA (F0), the offspring (F2) from F1 generation display a decrease of newly generated cells in the hippocampi of F2 female mice. BPA adversely affects hippocampal neurogenesis of future generation by modulating ERK and BDNF-CREB signaling cascades [13]. The fact that the second or third generation of mice shows epigenetic alterations even without any contact with BPA is important for the potential development of pathologies of BPA-treated patients.

13.2.4.2 Effects on Type 2 Diabetes

- Short-term treatment with BPA leads to metabolic abnormalities in insulin-sensitive peripheral tissues. Mice treated with BPA were insulin resistant and had increased glucose-stimulated insulin release. It was concluded that short-term treatment with low dose of BPA slows down whole body energy and disrupts insulin signaling in peripheral tissues. Therefore, BPA can be considered as a risk factor for the development of type 2 diabetes [14].

13.2.4.3 Obesity

- Exposure of 3T3-L1 preadipocytes for 14 days to BPA reduced the amount of triglyceride accumulation and suppressed the gene transcription of the lipogenic enzyme lipoprotein lipase. BPA can reduce triglyceride accumulation during adipogenesis [15].

13.2.4.4 Transgenerational Actions of Environmental Compounds

- After transient exposure of F0 gestating female rats during the period of embryonic gonadal sex determination, the subsequent F1–F3 generations were obtained in the absence of any environmental exposure. Spermatogenesis cell apoptosis was affected transgenerationally. Ovarian primordial follicle pool size was significantly decreased. Different DNA methylation of the F3 generation supports the altered epigenetic transgenerational inheritance [16].

13.3 Bisphenol A in Dental Materials

Since 40 years the pulp response to bisphenol A-releasing restorative materials was investigated. Comparison was made between a methyl methacrylate monomer and a dimethacrylate thinner material [17]. Two materials (a cement and a composite resin) were evaluated. The pulp reaction shows that they were within the limits of tolerance. After a strong initial response seen for the two materials, the long-term response (45 days) showed a well-defined repair and regeneration of the underlying pulp tissue.

One of the comonomer used to decrease the viscosity of the monomer forming the backbone of the composites is the bisphenol A-diglycidylether methacrylate (Bis-GMA). Dental products release BPA in some very particular conditions. Triethyleneglycol-dimethacrylate (TEGDMA) is ended by two functional methacrylate groups. Between the two methacrylate groups, the molecule is linear. The proportions of Bis-GMA and TEGDMA vary among the different products. BPA does not exist as such in composites, adhesives, or sealants, but is used in the synthesis of the main backbone molecule of the composite resins [18]. The existence of three different chemical forms has been reported: (1) the bisphenol A-diglycidylether methacrylate (Bis-GMA), (2) the monomer-like bisphenol A-diglycidylether (BADGE), and (3) the bisphenol A-dimethacrylate (Bis-DMA), used in some adhesives and sealants. The polymerization of the monomers and comonomers is never complete. The conversion rate (proportion of polymerized molecules compared to the initial amount of unpolymerized molecules) varies between 30 and 80 %, depending on the resins. However, there are always free monomers and comonomers released from a composite resin. Nevertheless, it is highly unlikely that TEGDMA can be produced by degradation of the polymerized matrix. Concerning BPA it is mostly found as an impurity in BADGE and Bis-DMA, which have less estrogenicity than the resins containing bisphenol A [19].

BPA is released from dental resins through salivary enzymatic hydrolysis of BPA derivatives. BPA is detectable in saliva for up to 3 h after resin placement. The majority of dental composites release TEGDMA in vitro and in vivo. This component is toxic. The compound induces allergies and cytotoxicity. Many reports describe allergic dermatitis in dental personnel, but far less in the oral cavity of patients. In an estrogen-sensitive cell line, estrogenic effects were found with BPA, Bis-DMA, and Bis-GMA, but not with TEGDMA.

Unpolymerized monomeric resin components from dental composites act on the function of accessory cells derived from the rat incisor pulp. Accessory cells and T lymphocytes reacted to low concentrations of urethane dimethacrylate, bis-glycidyl methacrylate, triethylene glycol dimethacrylate, and bisphenol A. They increased spleen cell proliferation to concanavalin A [20]. Transdentinal diffusion of BPA released from the resin composite restoration may demonstrate adverse effects on the dental pulp.

In vitro the resin component BPA was acting on the viability and substrate adherence capacity of macrophages. Viability was determined by trypan blue exclusion. The adherence index of macrophage decreased in the presence of 10^{-8} M BPA. It was concluded that the resin component BPA has the capacity to inhibit macrophage function and modulate immune and inflammatory response in dental pulp and periapical tissues [21].

13.4 Genetic and Cellular Toxicology of Dental Resin Monomers

Monomers cause adverse biological effects in mammalian cells. TEGDMA causes gene mutations in vitro. The formation of micronuclei indicates chromosomal damages, and monomers such as TEGDMA and HEMA induce DNA strand breaks. The comet assay quantified the DNA single-strand breaks, alkali labile, and incomplete excision repair sites [22]. The impairment of cellular pro- and antioxidant redox balance is caused by monomers. Monomers reduced the level of the radical scavenger glutathione (GSH) that protects the cells against reactive oxygen species (ROS). Cytotoxic and genotoxic effects of TEGDMA and HEMA are inhibited by the presence of ROS scavengers like N-acetylcysteine (NAC), ascorbate, and Trolox (vitamin E). Pathways regulating cellular homeostasis, dentinogenesis, or tissue repair may be modified by monomers at very low concentrations, in any case below those that induce acute toxicity [23].

13.4.1 Effects on Human Dental Pulp of Adhesive Resins and Monomers

Direct pulp capping increases the blood vessel density near the pulp exposure. VEGF

expression was upregulated primarily at post-transcriptional level [24].

There was a concentration-dependent decrease in cell proliferation and an increase in cell number after exposure to Bis-GMA. Cells showed typical characteristics of apoptotic cells after exposure to high concentrations of Bis-GMA. In contrast, cells exposed to low concentrations recovered their viability [25].

13.5 Summary

Bisphenol A is released by many resin components, including restorative materials, pit and fissure sealants, and resins aiming to seal orthodontic appliances. In vitro and in vivo adverse effects have been noted and the severity of these effects has been evaluated. A few toxic, genotoxic, and allergic reactions have been shown, displaying minor to severe responses. Many health concerns are documented, with increasing severity. During rodent development, cardiovascular, brain, and developmental deficiencies; obesity; and adverse effects of BPA have been well documented. In adult animals, as well as in humans, severe pathologies have been identified, such as diabetes, defective male and female genital tracts, ovarian cysts, and/or precancerous and cancerous lesions. However, it is difficult to extrapolate from animal pathologies to human. Therefore, the question of the potential adverse effects of resins releasing BPA remains open. Although some answers deny any adverse effects on public health in view of the small quantities released by BPA from restorative resins or sealants, the level being below the "non-detectable adverse effect level," four issues raise new insights and lead to a reappraisal of the safety of BPA in dentistry:

- Firstly, for a long period of time, the dose level was the most important point. Above a certain level, BPA was considered as a potential inducer of adverse effects. It is now clear that noxious effects are detectable even below a very low dose.
- Secondly, BPA effects appear to be transgenerational and they are observed even at the third generation of animals that have never received directly BPA. This means that our BPA-treated patients may not present immediately adverse effects. Two generations later, epigenetic effects might appear and noxious effects might influence some induced pathologies appearing at the third generation or later, even in BPA-untreated patients.
- Thirdly, the level of BPA in blood and urine levels, which was systematically measured up to now, may not be significant. In contrast, sweat analysis seems to provide more indicative information.
- Fourthly, it was recently shown that the possible high systemic bioavailability of BPA (70–90 %) is controlled by sublingual supervision. Along this line, the transmucosal absorption of BPA within the oral cavity led to much higher BPA internal exposure than the absorption resulting from the gastrointestinal tract. This focuses on the responsibility of BPA released from dental restorative material. The absorption through the oral mucosa may be an efficient systemic entry route, more efficient than orogastric gavage [26].

Altogether, these four points lead to reformulate the crucial question of the safety of BPA used in dentistry.

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