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Bisphenol A (BPA) the mighty and the mutagenic

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ABSTRACT

Bisphenol A (BPA) is one of the most widely used synthetic compounds on the planet. Upon entering the diet, its highest concentration (1–104 ng/g of tissue) has been recorded in the placenta and fetus. This accumulation of BPA can have many health hazards ranging from the easy to repair single strand DNA breaks (SSBs) to error prone double strand DNA breaks (DSBs). Although the Human liver can efficiently metabolize BPA via glucuronidation and sulfation pathways, however the by-product Bisphenol-*o*-quinone has been shown to act as a DNA adduct. Low doses of BPA have also been shown to interact with various signaling pathways to disrupt normal downstream signaling. Analysis has been made on how BPA could interact with several signaling pathways such as NFκB, JNK, MAPK, ER and AR that eventually lead to disease morphology and even tumorigenesis. The role of low dose BPA is also discussed in dysregulating Ca^{2+} homeostasis of the cell by inhibiting calcium channels such as SPCA1/2 to suggest a new direction for future research in the realms of BPA induced disease morphology and mutagenicity.

1. Introduction

BPA is a synthetic phenol extensively used in the manufacture of polycarbonate plastics and epoxy resins. It was first reported by a Russian chemist Aleksandr Dianin in 1891 [1] (Fig. 1) and synthesized via the condensation of acetone with two equivalents of phenol by Zincke in 1905 [2].

Although BPA is one of the most widely used synthetic compounds on the planet with an annual production of about 5 million tonnes in the United States, but the biggest growth is being observed in Asia with 13% annual average growth and 19% growth in the demand of polycarbonates in India [3]. A worrisome effect of BPA is that it leaches out from the food and beverage containers that are manufactured by using BPA and leaches into the contents [4–7]. Human BPA exposure through consumption of canned food has been estimated to be 6.6 μg/person/day [8] which then enters the blood stream [9,11,12]. Analysis of urine samples of co-habitating male and female partners were correlated indicating a common exposure source of BPA [13]. With a minimum detection limit of 0.4 μg/L urine, BPA was detected in 92.6% of the samples examined. The mean value reported was 2.6 μg/L (2.6 μg/g creatinine) and the 95th percentile concentration of 15.9 μg/L (11.2 μg/g creatinine). Of the 2517 participants, females had statistically higher BPA concentrations than males ($p = 0.043$) and children had higher concentrations than adolescents ($p < 0.001$) whereas adolescents had higher concentrations than adults ($p = 0.003$). Urine concentrations were linked to race/ethnicity, age, gender and even the household income [14]. Quantification of BPA in sweat via solid phase extraction (SPE) followed by analysis with HPLC has shown that BPA could be recorded in 16 of the 20 samples. Moreover, the team was careful in their sample collection methodology for directly transferring sweat from skin to the glass containers using stainless steel spatulae [15]. In a similar study conducted in 2015 the amount of BPA excreted in sweat of

Abbreviations: GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; ELISA, enzyme linked immunosorbent assay; MS, mass spectrometry; LLE, liquid/liquid extraction; SPE, solid phase extraction; DES, diethyl stilbesterol; SPCA1, secretory pathway calcium ATPase1; IGF1R, insulin-like growth factor 1 receptor; FDA, Food and Drugs Administration; EFSA, European Food Safety Authority; FAO/WHO, Food and Agricultural Organization/World Health Organization

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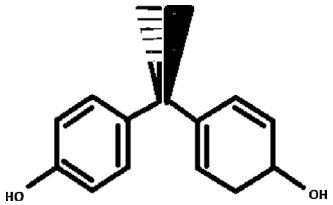

Fig. 1. Bisphenol A, molecular formula: $C_{15}H_{16}O_2$, molar mass is 228.29 g/mol.

50 subjects was attempted but used polyurethane sweat patches to later extract BPA by placing the patches in polypropylene plastic tubes. The team reported five-fold higher BPA in sweat than the background [16]. Further, this high concentration of BPA showed up only in 3 out of 50 samples. However, the experimental design and sample sweat collection methodology both need modification as the use of any kind of plastics in BPA biomonitoring could be severely undermined due to external contamination. Isocyanate based polyurethanes raise severe toxicity concerns, however non-isocyanate polyurethanes have also been developed [17]. Biomonitoring of BPA in 69 hair samples collected from urban and rural Greek population was evaluated using LCMS analysis. The results showed that 41.2% of urban samples had a mean BPA burden of 64.1 pg mg^{-1} while only 14.8% of the rural samples showed BPA at a mean concentration of 40.3 pg mg^{-1} [18]. These observations are a clear evidence of the link between lifestyle exposure of BPA versus detection in human blood, urine or sweat.

Reported occurrence of BPA in baby bottles raised an alarm in 2003 [9] and was also reported from the animal cages that were manufactured using BPA containing polycarbonates [10]. Factors such as heat, acidic or basic pH levels were also indicted in enhanced leaching of BPA from the containers [12]. Occurrence of BPA in free and conjugated forms was validated in blood of pregnant women and the highest concentration was measured in placenta and the developing fetus [18]. This accumulation can lead to birth defects as shown in animal studies [22]. Human epidemiological studies also reveal the relationship between BPA exposure and repeated miscarriage [23]. Concentrations of 0.3–18.9 ng/mL were measured in maternal plasma and 1–104.4 ng/g in the placental tissue and male fetuses accumulated significantly more BPA than female fetuses. Passage of BPA across the placenta has also been reported and found to in the range of 1–18 ng/mL in the maternal serum, 1–10 ng/mL in amniotic fluid and cord serum at birth and up to 100 ng/g in placenta [19,20]. However the concentration of BPA in human serum is about 0.1–1000 nM [21]. Besides serum, blood and placenta; BPA has also been detected in the milk of healthy women at a concentration of 0.28–0.97 ng/mL [25]. The objective of this study was to explore the possible molecular mechanisms that drive toxicity build-up in humans that affect DNA and signaling pathways in different ways that can initiate tumorigenic events.

2. Quantification of BPA in body fluids

Different analytical techniques have been used since 1999 to measure unconjugated BPA concentrations in human serum at levels ranging from 0.2–20 ng/mL and exceeding 100 ng/g in a study that focused on toxicity in placental tissue [18]. Some of the techniques employed for this purpose are gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA). Several analytical techniques including the highly sensitive mass spectrometry (MS), specifically isotope dilution-MS, is considered a relatively reliable method for measuring trace levels of BPA and other environmental chemicals in biological samples [24]. The validity of methods used by Schönfelder et al. for BPA quantification in human fluids and use of standard BPA free controls has been debated widely but arguments provided by Völkel et al. relate high BPA urine concentrations to external contamination, sampling error or insensitive analytical methods. But an alternative explanation to the occurrence of free/unconjugated, conjugated or substituted BPA reported in several studies conducted on human blood, serum and the placental tissue was not provided [26]. Furthermore, oral pharmacokinetic studies in rodents and rhesus monkeys using isotopically labeled BPA indicate that intakes of 75 to over 1000 μg/kg BW/day would be required for the high levels of unconjugated BPA reported in the human literature assuming similar kinetic parameters [27]. Thus more sensitive methods were required to determine free/unconjugated BPA in human fluids.

As BPA enters the blood through diet it can be effectively detoxified through glucuronidation and sulfation [45] (please see BPA metabolism in mammals) but until the detoxification process begins the free/unconjugated form of BPA can exist in blood and serum for almost 1–2 h after one oral dose of 100 μg/kg BW of deuterated BPA (d6-BPA) by oral administration. Unconjugated d6-BPA was detected in serum within 5–20 min of oral dosing with a mean C_{max} of 6.5 nM (1.5 ng/mL) observed at T_{max} of 1.3 ± 0.52 h. Detectable blood levels of unconjugated BPA (d6-BPA) could be measured for up to 48 h [28]. BPA can be conjugated to glucuronide (BPAG) and eliminated through urine [28,29] but can be deconjugated via β-glucuronidase which is present at high concentrations in liver, kidney, intestine and placenta [30]. The deconjugation of BPAG to BPA increases the potential reactivation of BPA induced effects. A new method for the determination of free BPA, BPAG, BPA disulfates (BPADS) and three BPA chlorides, namely BPA mono-(BPAMC), di-(BPADC), trichloride (BPATrC), in human urine and serum samples, using solid-phase extraction (SPE) and LC–MS/MS detection was described recently and attempted to eliminate possible exogenous BPA contamination through the use of glass apparatus, instead of plastic tubes, for sample preparation and washed their apparatus with formic acid. Internal standard for establishing baseline for any possible BPA contamination was used besides verifying instrumental calibration by injecting 10 μL of 0.01–100 ng/mL standards of the target compounds, that demonstrated excellent linearity (regression coefficient = $r > 0.99$). Upon comparison of two methods (liquid/liquid extraction LLE versus solid phase extraction SPE) the values for conjugated and unconjugated forms of BPA were measured and reported to be 5 times higher in urine using SPE method than measured via LLE method. However, the concentrations of BPA in serum were not significantly different than reported in earlier investigations or LLE method [31]. Other studies also confirm the unconjugated occurrence of BPA in blood and serum even when all possible precautions were taken to avoid external contamination [32].

BPA has been recently reclassified as a class 1B reproductive toxin by the European chemical classification and labeling (CLP) [34] and a similar essential component of epoxy resins called bisphenol A diglycidyl ether, to which humans are widely exposed, was previously classified as a suspected class 2 B carcinogen by the International agency for research on cancers (IARC) [33]. It is noted that toxic exposure data for humans is missing but it was shown to be carcinogenic in animal models. It is also claimed by IARC that glycidaldehyde, a metabolite of bis-phenol A diglycidyl ether, is carcinogenic to experimental animals and classified as possibly carcinogenic to humans (group 2B) in their 1989 report [33].

North America and Europe have taken steps to either ban the use of BPA or shown concern vis-à-vis its use in food containers. Canada became the first Country to ban BPA in October 2008 through its chemicals management plan [35]. Other Countries have issued exposure warnings but not banned the compound due to insufficient direct link between dose and incidence. Asia in general and South Asia in particular has only recently realized the health hazards posed by the biotoxic nature of BPA.

Earlier studies of Food and Drugs Administration (FDA) in 2008 [36,37], based on available research data, but no new research, suggested that BPA is not toxic for humans, even for unborn babies. The European Food safety Authority (EFSA) issued a new tolerable daily intake (TDI) of 4 μg/kg of bw/day of BPA in 2011, which significantly