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Genotoxic potentials and related mechanisms of bisphenol A and other bisphenol compounds: A comparison study employing chicken DT40 cells

Sangwoo Lee [a], Xiaoshan Liu [a], Shunichi Takeda [b], Kyungho Choi [a,*]

[a] School of Public Health, Seoul National University, Seoul 151-742, Republic of Korea
[b] Department of Radiation Genetics, Graduate school of Medicine, Kyoto University, Kyoto 606-8501, Japan

HIGHLIGHTS

- Bisphenol A and alternative bisphenols showed genotoxic potentials in DT40 cells.
- Their mechanisms involve double strand breaks rescued by homologous recombination.
- *RAD54*$^{-/-}$ DT40 mutant cells were the most hypersensitive to most bisphenols.
- Genotoxic potential was confirmed by chromosomal aberration and γ-H2AX foci.

GRAPHICAL ABSTRACT

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ABSTRACT

Bisphenol A (BPA) has been found in plastic food containers, paper currencies and toys. BPA has been reported for various adverse health concerns including reproduction, development and carcinogenesis. These potential health implications have led to increasing use of alternative bisphenols such as bisphenol F and bisphenol S among many. However, little is known about the toxicity of alternative bisphenols and most of the toxicological information is limited to endocrine disrupting potentials. In this study, we evaluated cytotoxicity and the genotoxic potentials of several bisphenol compounds, and identified the mechanism of genotoxicity using a panel of mutant chicken DT40 cell lines deficient in DNA repair pathways. Several bisphenols including bisphenol AP, bisphenol M, or bisphenol P exerted genotoxic potentials that are greater than that of BPA. Generally *RAD54*$^{-/-}$ mutant cells were the most sensitive to all bisphenols except for bisphenol F, suggesting the induction of DNA double-strand breaks that could be rescued by homologous recombination. Genotoxic potential of bisphenols was confirmed by chromosomal aberration assay and γ-H2AX foci forming assay between *wild-type* and *RAD54*$^{-/-}$ mutant. Among the tested bisphenols, BPP at 12.5 μM showed the greatest genotoxic potency, inducing chromosomal aberration and γ-H2AX foci in *RAD54*$^{-/-}$ mutant by 2.6 and 4.8 folds greater than those in *wild-type*, respectively. Our results clearly show several alternative bisphenols can cause genotoxicity that could be rescued by homologous recombination pathway, and some bisphenols induced even greater genotoxic potentials than that of BPA.

* Corresponding author. Address: School of Public Health, Seoul National University, Gwanak, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 2738; fax: +82 2 745 9104.
E-mail address: Kyungho@snu.ac.kr (K. Choi).

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1. Introduction

Bisphenol analogues, including bisphenol A (2,2-bis-(4-hydroxyphenyl)propane; BPA) are used in various industrial and commercial applications. BPA has been most widely used in polycarbonates and epoxy resins. BPA has also been detected in plastic food containers, thermographic and pressure sensitive papers, paper currencies, toys, medical tubing, and dental fillings (Bailin et al., 2008; Huang et al., 2012; Liao et al., 2012a,b). The amount of global BPA production was projected at 4.0 million metric tons by 2011 (Bailin et al., 2008). Widespread use of BPA in many applications has resulted in its detection in dietary items (Schecter et al., 2010) and human biological samples (Sun et al., 2004; Calafat et al., 2005; Liao et al., 2012a). BPA was detected in environmental media as well (Huang et al., 2012). Its concentrations were generally lower than 1 $\mu g\,L^{-1}$ in aquatic environment, however often two to three orders of magnitude higher in industrial effluents (Fukazawa et al., 2001). In addition, BPA has been reported in soil and air samples (Khim et al., 2001; Fu and Kawamura, 2010). In fish liver, BPA was detected at levels between 2 and 75 ng g^{-1} dry weight in the Netherlands (Belfroid et al., 2002). In squid, BPA has been detected on average 257.3 ng g^{-1} with 76.5% of detection frequency in Malaysia (Santhi et al., 2012).

Exposure to BPA has been associated with various health effects including reproduction and development related implications and carcinogenesis, although its low-dose toxicity is still controversial (Chen et al., 2002; Zhang et al., 2011). Recent studies showed that BPA binds with estrogen receptor (Okada et al., 2008), and disrupts steroidogenic potential in human cell (Zhang et al., 2011). BPA exposure resulted in increase of tail moment and tail length in comet assay (Ulutas et al., 2011), suggesting mutagenic and genotoxic potentials. Due to such potential health implications, the use of BPA in infant feeding bottles has been banned in 2011 in Europe (EFSA, 2006) and Canada (Health Canada, 2009). Such restrictions along with increased public concerns have led to increased use of alternative bisphenols. Such alternatives include bisphenol AF (BPAF; 2,2-Bis(4-hydroxyphenyl)hexafluoropropane), bisphenol F (BPF; 4-hydroxyphenyl)methane), and bisphenol S (BPS; 4-hydroxyphenyl)sulfone), among many (Liao et al., 2012a). According to the Inventory Update Rule (IUR) production volume list, approximately 10 000–500 000 lb of BPAF and 1 000 000–10 000 000 lb of BPS were produced annually by 2002 (NTP, 2008).

While comprehensive information is available about the adverse health impacts of BPA, toxicological properties of alternative bisphenols are yet to be investigated. Alternative bisphenols are structurally similar to BPA (Chen et al., 2002), and therefore expected to possess similar biological activities. Most available toxicological information is limited to endocrine disrupting potentials (Chen et al., 2002), and only very little is known about the genotoxicity of alternative bisphenols. BPA is considered to lead to genotoxicity through oxidative stress. Bisphenol A-3,4-quinone (BPAQ), which is yielded by oxidative metabolism of BPA, may cause genotoxicity by reacting with DNA (Kolšek et al., 2012, 2013). BPA may cause oxidative stress, and induce DNA adduct and aneuploidy in rodents (Tiwari et al., 2012). However, Chen et al. (2002) reported that eight bisphenols including BPA showed no positive responses based on *umu*-test suggesting no genotoxicity. Similarly, other alternative bisphenols are expected but to date very little efforts have been made on this aspect. In the present study, we investigated the genotoxicity potential and relative mechanisms of several bisphenols using a panel of genetically modified chicken DT40 cells. The chicken DT40 cell has been used to sensitively identify genotoxicity of chemicals and environmental samples in mechanism specific way (Hu et al., 2012). The results of this study will help understand potential health implications of BPA and other alternative bisphenols, and can be employed to develop safer alternatives.

2. Materials and methods

2.1. Test chemical preparation

Eight bisphenol chemicals, i.e., BPA, BPAF, bisphenol AP (BPAP; 4,4′-(1-Phenylethylidene) bisphenol), bisphenol C (BPC;2,2-Bis(4-hydroxy-3-methylphenyl) propane), BPF, bisphenol M (BPM; 4,4′-(1,3-Phenylenediisopropylidene) bisphenol), bisphenol P (BPP; 4,4′-(1,4-Phenylenediisopropylidene) bisphenol), BPS were purchased from Sigma Aldrich (St. Louis, MO, USA) (Table 1). All bisphenols were dissolved in dimethyl sulfoxide (DMSO), and the maximum volume of the solvent did not exceed 1.0% v/v. Experimental doses for DT40 cell bioassay for each test compound were chosen based on preliminary range-finding tests using *wild-type* DT40 cells. Positive controls were not included in the present study but the responses of the DT40 cell clones used here against several known genotoxicants have been reported previously by our group (Ji et al., 2009, 2011a; Liu et al., 2012).

2.2. DT40 cell lines and culture

A panel of isogenic DT40 mutants such as *Ku70*$^{-/-}$, *Polβ*$^{-/-}$, *RAD54*$^{-/-}$, *REV3*$^{-/-}$, and *XPA*$^{-/}$, each defective in one of the major DNA damage repair mechanisms, was employed. These repair pathways include base excision repair (*Polβ*$^{-/-}$) (Tano et al., 2007), nucleotide excision repair (*XPA*$^{-/}$) (Kelsall et al., 2012), homologous recombination (*RAD54*$^{-/-}$) (Bezzubova et al., 1997), non-homologous end-joining (*Ku70*$^{-/-}$) (Takata et al., 1998), and translesion DNA synthesis (*REV3*$^{-/-}$) (Sonoda et al., 2003). The mutant cell lines were developed and successfully demonstrated for the utility in screening and characterizing genotoxicity of environmental contaminants elsewhere (Ji et al., 2009, 2011a; Evans et al., 2010; Ridpath et al., 2011; Yamamoto et al., 2011; Hu et al., 2012). Cells (1×10^5) were cultured in 100 mm Petri-dishes with 10 mL RPMI 1640 medium (Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO), 1% chicken serum (Sigma), 1% L-glutamin-200 mM stock solution (Invitrogen, Carlsbad, CA, USA), 1% penicillin/streptomycin (Sigma), and 50 μM β-mercaptoethanol (Sigma) at 39.5 °C in a humidified atmosphere of 5% CO_2 and 95% air (Sanyo, Osaka, Japan).

2.3. Adenosine-5′-triphosphate (ATP) assay

In order to differentiate genotoxicity from cytotoxicity, only doses that resulted in <20% decrease of cell proliferation in *wild-type* cells were considered as the 'non-cytotoxic' dose. When the cell viability of a given mutant was decreased by >3-fold compared to that of the *wild-type* after exposure to the non-cytotoxic dose, the mutant was determined as hypersensitive, and the given test compound was classified as genotoxic (Ji et al., 2011b). Based on this approach, genotoxicity of bisphenols can be detected by simply monitoring the differences in cellular proliferation rates between *wild-type* cells and the isogenic clones deficient in specific DNA repair pathways at certain doses (Ji et al., 2009, 2011a; Liu et al., 2012). Cells (~5000 cells) in 100 μL/well of culture medium were seeded into 96-well plates, and were exposed to various concentrations of target bisphenols for 48 h. ATP assays were carried out with 96-well plates using a CellTiter-Glo® Luminescent Cell Viability Assay Kit (Promege Corp., Madison, WI, USA), and luminescence measured by use of a Tecan infinite® 200 (Tecan Group