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In vitro and in vivo estrogenic activity of chlorinated derivatives of bisphenol A

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Abstract

The estrogenic activity of bisphenol A (BPA) and its chlorinated derivatives, 2-(3-chloro-4-hydroxyphenyl)-2-(4-hydroxyphenyl)propane (3-ClBPA) and 2,2-bis(3-chloro-4-hydroxyphenyl)propane (3,3′-diClBPA) was assessed by determining their relative binding affinity for the human estrogen receptor-α and -β (ERα and ERβ) and also their uterotrophic activity in ovariectomized female rats. BPA and its chlorinated derivatives were active in competing with [^{3}H]17β-estradiol for their binding to the human ERα and ERβ proteins. While 3-ClBPA and 3,3′-diClBPA competed more effectively for ERα binding than BPA (IC_{50} values of 2.48×10^{-5}, 1.28×10^{-5}, and 1.08×10^{-4} M, respectively), they had similar activity as BPA for competing the binding to ERβ (IC_{50} values of 1.43×10^{-5}, 1.87×10^{-5}, and 2.59×10^{-5} M, respectively). To determine the uterotropic activity, three doses (10, 50 and 100 mg/kg/day) of BPA and its derivatives were given to mature ovariectomized Sprague–Dawley rats for 3 consecutive days. Treatment of animals with 50 and 100 mg/kg/day of BPA or its chlorinated derivatives caused a significant increase in the uterine wet weight and the endometrial area. The results of our present study demonstrated that the affinities of 3-ClBPA and 3,3′-diClBPA for ERα were higher than the affinity of BPA, although the in vivo estrogenic activity of the two chlorinated BPAs in ovariectomized female Sprague–Dawley rats appeared to be comparable to that of BPA.

Keywords: Chlorinated bisphenol A; Environmental estrogens; Estrogen receptor; Uterotrophic assay

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1. Introduction

Bisphenol A (2,2-bis[4-hydroxyphenyl]propane, abbreviated as BPA) is widely used in industries of the polymer and polycarbonate plastics. In addition to its wide use in the production of various types of papers (e.g., thermal paper and carbonless copy paper), BPA is also used as one of the basic elements for the food container linings, dental sealants, and many household plastic products and food/drink-packaging materials (Staples et al., 1998).

Notably, BPA was among the first group of the identified endocrine disrupting chemicals (EDCs) (Dodds and Lawson, 1938). This EDC has been shown to promote the proliferation of cultured human MCF-7 breast cancer cells (Krishnan et al., 1993) and bind to the estrogen receptor (Matthews et al., 2001). Gaido et al. (1997) reported that BPA activates the expression of the estrogen response element (ERE)-driven reporter gene constructs. Additional studies using immature or ovariectomized mature rodents have shown that BPA has estrogenic activity in vivo by increasing the uterine wet weight (Ashby and Tinwell, 1998; Yamasaki et al., 2000; Papaconstantinou et al., 2000; Kim et al., 2001).

BPA is readily chlorinated by reactions with sodium hypochlorite, which is commonly applied as a bleaching agent in paper factories and also as a disinfectant in sewage treatment plants. Chlorinated derivatives of BPA such as 2-(3-chloro-4-hydroxyphenyl)-2-(4-hydroxyphenyl)propane (3-ClBPA) and 2,2-bis(3-chloro-4-hydroxyphenyl)propane (3,3′-diClBPA), structured shown in Fig. 1, have been detected in final effluents of paper manufacturing plants (Fukazawa et al., 2001). The estrogenic activity of these compounds has been evaluated using the yeast two-hybrid system and the green fluorescent protein (GFP) expression system in MCF-7 cells. All of the chlorinated derivatives were reported to have more potent estrogenic activity than BPA in the yeast two-hybrid assay (Fukazawa et al., 2002). In the GFP expression system, 3-ClBPA and 3,3′-diClBPA exhibited a similar estrogenic activity, and both of them appeared to be more potent than their parent compound BPA (Kuruto-Niwa et al., 2002). However, very few studies have examined the in vivo estrogenic activity of the chlorinated BPA derivatives. In the present study, therefore, we compared the in vitro and in vivo estrogenic activity of 3-ClBPA and 3,3′-diClBPA with BPA by determining their relative binding activity for the human ERα and ERβ and also by determining their uterotrophic activity in ovariectomized female SD rats.

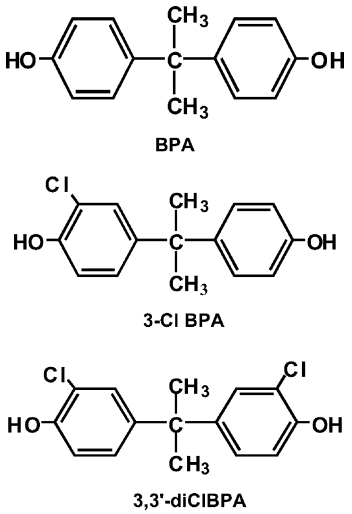

Fig. 1. Chemical structures of BPA and its chlorinated derivatives.

2. Materials and methods

2.1. Chemicals

BPA was purchased from Kanto Chemical Co., Tokyo, Japan. 3-ClBPA and 3,3′-diClBPA were chemically synthesized and purified as previously reported (Fukazawa et al., 2001). The recombinant human ERα and ERβ proteins were obtained from PanVera Corporation (Madison, WI). Hydroxylapatite (HAP) came from Calbiochem (San Diego, CA). [2,4,6,7,16,17-^{3}H]E_2 and 5-bromo-2′-deoxyuridine (BrdU) were obtained from NEN Life Sciences (Boston, MA) and Sigma Chemical Co. (St. Louis, MO), respectively.

2.2. Animals

Female Jcl: Sprague–Dawley rats, 6- or 7-weeks old (150 ± 50 g), were purchased from SLC Japan Inc., Tokyo, Japan. Rats were housed in multiple rat racks suitable for this animal strain and had free access to the NIH-07 phytoestrogen-low diet (Oriental Yeast Co., Tokyo, Japan) and tap water. The animal holding