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Growth-promoting effect of bisphenol A on neuroblastoma in vitro and in vivo☆

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Abstract

Purpose: To investigate the effect and mechanism of bisphenol A (BPA), one of the main environmental endocrine disruptors, on the proliferation of human neuroblastoma cells.

Methods: In vitro, cultured SK-N-SH cells were treated with 17β-estradiol (E_2; 1 ng/mL), BPA (2 μg/mL) with or without estrogen receptor antagonist ICI182,780 (10^{-6} mol/L). Viable cell number, DNA proliferation index, and expression of cyclin-dependent kinase 4 and cyclin D1 were assessed by absorbance reading, flow cytometry, and western blotting, respectively. In vivo, ovariectomized nude mice bearing SK-N-SH tumors were administered by gavage with E_2 (500 μg/kg per day, n = 11), BPA (200 mg/kg per day, n = 10), or vehicle (n = 9) for 18 days. Mice body weight, tumor volume and weight were examined every 3 days. Tumor microvessel density, proliferating cell nuclear antigen and vascular endothelial growth factor expression were evaluated by immunohistochemical staining or western blotting.

Results: In vitro, the BPA group had 20% higher number of viable cells, 70% higher proliferation index (both $P < .01$), and higher expression of cyclin-dependent kinase 4 and cyclin D1 than the nontreated group. In vivo, the BPA group had over 50% higher gross tumor volume, tumor weight, microvessel density, proliferating cell nuclear antigen ($P < .05$ or .01), and higher vascular endothelial growth factor protein expression than the mock control group. Both in vitro and in vivo BPA effects were comparable with those by E_2. ICI182,780 effectively abolished the promoting effect for both.

Conclusions: Bisphenol A can promote the growth of neuroblastoma to a level similar to that of E_2. Estrogen receptor–dependent pathway and angiogenesis may contribute to the underlying mechanisms.
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Since the 1960s, there has been a growing concern on environmental contamination by chemical agents produced by industrial, mining, and agricultural activities, such as pesticides, herbicides, fungicides, and plastic compounds. Such compounds interfere with appropriate sex steroid receptor action and, thus, are referred to as environmental

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endocrine disruptors (EED), also called environmental estrogens or xenoestrogens. Environmental endocrine disruptors are exogenous agents that interfere with the synthesis, secretion, and function of hormones within the body which are responsible for the maintenance of homeostasis, reproduction, development, and behavior [1]. Environmental factors have been implicated as causative factors for many human diseases such as deformity, reproductive disturbances, and breast cancer. Children are especially vulnerable to EED exposure because of their physiological characteristics [2]. Based on consistent epidemiological studies, the occurrence of childhood asthma, neurologic developmental disorders, hypospadias, and early puberty has increased in the last 10 years [2]. The increasing incidence of childhood cancer such as acute lymphoblastic leukemia, brain cancer, and testicular cancer is believed to be related to the presence of chemical agents in the environment [3,4]. Bisphenol A (BPA), one of the most important EED, is widely used as a material for the production of epoxy resins and polycarbonate plastics. With increased use of plastic products, human exposure to BPA has increased. Perinatal exposure to BPA results in morphological and functional alterations of the male and female genital tract and glands that may predispose the tissue to earlier onset of diseases, lower fertility, and cancer such as breast cancer and prostate cancer [5-7]. However, the mechanisms of BPA actions are poorly understood. Previous studies have indicated that BPA exerts estrogenic effects through estrogen receptor (ER), which has been confirmed to be closely related to endocrine tumors such as breast cancer [8]. ICI182,780 is a pure ER antagonist that binds ER specifically and results in its degradation, thereby blocking ER-dependent biologic effects. Known as Fulvestrant in the clinic, ICI182,780 is currently applied in the treatment of ER-positive advanced or metastatic breast cancer as an alternative for tamoxifen. In contrast to tamoxifen, fulvestrant has no known estrogen agonist effects and thus provides an effective and well-tolerated option for the treatment of patients with breast cancer [9].

Neuroblastoma is a common pediatric cancer. It arises from neural crest cells and is most commonly found in the adrenal medulla or along the sympathetic chain. It accounts for over 7% of malignancies in patients younger than 15 years of age and about 15% of all pediatric oncology deaths [10]. However, neuroblastoma oncogenesis and its mechanisms are poorly understood. Therefore, prognosis of the advanced stage of disease still remains poor despite aggressive treatment regimens. In the past few years, the outcome for children with a high-risk clinical phenotype has improved only modestly, with long-term survival still less than 40% [11,12]. Therefore, researchers are striving to elucidate the pathogenesis of neuroblastoma with the goal of creating improved precautions and treatments for this type of tumor.

There are few reports concerning the effect of EED on human neuroblastoma cells and its molecular mechanisms. In this study, we investigated the effect of BPA on the proliferation of human neuroblastoma in vitro and in vivo. Parallel experiments were performed with 17β-estradiol (E_2) as the positive control.

1. Materials and methods

1.1. Cell culture

A neuroblastoma cell line, SK-N-SH (Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, China), was cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Scotland, UK) containing 10% heat-inactivated fetal bovine serum (FBS, Biochrom, Berlin, Germany), 100 U/mL penicillin G, 100 μg/mL streptomycin, and 0.1 mol/L L-glutamine. Cells were grown in a humidified atmosphere with 5% CO_2 in air at 37°C. Before treatments, cells were grown in phenol red-free RPMI-1640 medium (Gibco, Scotland, UK) supplemented with 10% charcoal-dextran-stripped FBS (cd-FBS, Gibco, Scotland, UK) for 48 hours [13].

1.2. Cell counting kit–8 assay

Cells were seeded into 96-well plates at a density of 1×10^4 per well in phenol red–free RPMI-1640 medium with 5% cd-FBS overnight. Cells were treated the following day with E_2 (1 ng/mL; Sigma-Aldrich, St Louis, Mo), BPA (2 μg/mL; Shanghai Chemical Reagents Company, Shanghai, China), or medium only with or without additive treatment of ER antagonist ICI182,780 (10^{-6} mol/L; Tocris, Bristol, UK).

The number of viable cells were quantified with the cell counting kit–8 assay (Dojindo Laboratory Co, Ltd, Kumamoto, Japan) [14] on day 0 (after treatment), day 2, and day 5. To each well in the assay plate, 10 μL of the cell counting kit solution was added, and then 2 hours of incubation at 37°C was carried out. The absorbance value (AV) was measured at 450 nm using a microplate reader (Model 680, Bio-Rad, Hercules, Calif) and used as a correlate of viable cell number. The treatment wells were in quadruplicates and the whole experiment was repeated 3 times.

1.3. Flow cytometry

Cells were cultured in serum-free medium for 48 hours, seeded in phenol red-free RPMI-1640 medium containing 5% cd-FBS and then treated as described above. After 5 days of drug exposure, cells were collected using 0.25% trypsin/0.02% EDTA and resuspended in cold phosphate-buffered saline at a concentration of 1×10^6 per milliliter. The cells were then fixed in citrate buffer for 30 minutes at room temperature and DNA was stained with propidium iodide containing 50 μg/mL RNase. Cell cycle analysis was

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