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Low-level bisphenol A increases production of glial fibrillary acidic protein in differentiating astrocyte progenitor cells through excessive STAT3 and Smad1 activation

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Abstract

The effects of bisphenol A (BPA) on the differentiation of serum-free mouse embryo (SFME) cells, the astrocyte progenitor cells in the central nervous system, were examined. SFME cells were exposed to 10 ng/ml leukemia inhibitory factor (LIF) and 10 ng/ml bone morphogenetic protein 2 (BMP2) to increase glial fibrillary acidic protein (GFAP) expression and induce cell differentiation. Various concentrations of BPA (0.1 pg/ml–1 μg/ml) were then added to determine their effects on the cell differentiation. SFME cells were effectively differentiated by LIF and BMP2 in completely serum-free cultures. Cell proliferation following cell differentiation was not significantly affected by low-level BPA. However, GFAP expression was significantly increased in SFME cells in the presence of 1–100 pg/ml BPA. These increases were due to excessive activation of signal transducer and activator of transcription 3 (STAT3) and mothers against *decapentaplegic* homolog 1 (Smad1) by the low-level BPA.
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1. Introduction

Bisphenol A (BPA) is an industrial chemical that serves as a raw material for the manufacture of a variety of compounds, many of which are present in a number of consumer products. Its major use is as a monomer in the production of polycarbonates and epoxy resins. BPA is also used in lesser amounts in various applications, for example, as an intermediary in thermal paper production and as an antioxidant and inhibitor of end polymerization in the manufacture of plastics. Since BPA is used in the manufacture of such a variety of consumer products, there are a number of possible routes of exposure for the general public, such as: (1) direct and indirect environmental exposure due to the release of BPA during its production, use and disposal; (2) exposure through leaching into food; (3) contact with or inhalation of non-food-contact consumer products. Environmental

Abbreviations: BMP2, bone morphogenetic protein 2; BPA, bisphenol A; CNS, central nervous system; DIC, differential interference contrast; ER, estrogen receptor; GFAP, glial fibrillary acidic protein; IgG_1, immunoglobulin G_1; LIF, leukemia inhibitory factor; OLIG2, oligodendrocytic basic helix–loop–helix transcription factor 2; SFME, serum-free mouse embryo; Smad mothers against *decapentaplegic* homolog; STAT, signal transducer and activator of transcription; TAM, tamoxifen; TBST, TBS containing 0.05% Tween-20

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measurements and knowledge of the properties of BPA suggest that environmental sources of BPA exposure do not contribute significantly to the overall population exposure (Kamrin, 2004). However, in light of reports suggesting that low doses of BPA cause estrogenic effects in laboratory animals (Nagel et al., 1997), concerns about the exposure from and safety of some consumer products, particularly those made from plastics, have been raised. Therefore, in order to evaluate the risk, if any, from BPA, investigations have been undertaken to study the validity of the reported low-dose effects more carefully.

Exposure to low doses of BPA during development caused adverse reproductive effects in rodents, such as increased prostate weight (Nagel et al., 1997) and decreased epididymal weight (Vom Saal et al., 1998). However, attempts to duplicate these results in other laboratories were unsuccessful (Ashby et al., 1999; Safe, 2000) and the public attention to these discrepancies was serious enough to constitute the formation of expert panels to review the available data in the USA in 2001 and the EU in 2003. One of the reasons for the dispute and concerns is that BPA could bind to estrogen receptors (ERs) and be capable of inducing ER-mediated gene expression with comparable efficacy (Matthews et al., 2001), thereby bringing about a higher incidence of endocrine-related diseases that are thought to be associated with exposure to environmental chemicals with estrogenic activity (Jones and Hajek, 1995). It has been demonstrated that BPA was able to reach the fetus in the womb (Takahashi and Oishi, 2000; Schönfelder et al., 2002) and that fetal exposure to low doses of BPA led to disruption of reproductive organs and their functions (Welshons et al., 1999; Akingbemi et al., 2004), implying that the perinatal period could be a very sensitive time for exposure to BPA. However, low doses of BPA over two or three generations did not cause significant compound-related adverse effects on reproductive parameters in rats (Ema et al., 2001; Tyl et al., 2002).

Although a variety of studies on reproductive parameters have been described, little attention has been directed toward the potential consequences of low-dose exposure of BPA for the development of the central nervous system (CNS). In the present study, serum-free mouse embryo (SFME) cells, the astrocyte progenitor cells, were exposed to LIF and BMP2 to induce differentiation in a completely serum-free culture system and morphological changes, cell proliferation and expression of glial fibrillary acidic protein (GFAP), the astrocyte marker protein, were analyzed. After the presence of ERs in the cells was determined, the effects of low-level BPA on cell proliferation, production of GFAP and activation of signal transducer and activator of transcription 3 (STAT3) and mothers against *decapentaplegic* homolog 1 (Smad1) were also analyzed in the differentiating SFME cells to investigate and predict its possible effects on differentiation of brain cells and development of brain.

2. Materials and methods

2.1. Materials

BPA, TAM and standard GFAP were purchased from GL Sciences Inc. (Tokyo, Japan), ICN Pharmaceuticals Inc. (Aurora, OH) and Cytoskeleton Inc. (Denver, CO), respectively. An anti-GFAP monoclonal antibody and AP-conjugated antibody against mouse immunoglobulin G_1 (IgG_1) were obtained from YLEM (Rome, Italy) and ICN Pharmaceuticals Inc. (Aurora, OH), respectively. An anti-ERα polyclonal antibody, anti-ERβ monoclonal antibody, anti-pSTAT3 polyclonal antibody, anti-pSmad1 polyclonal antibody, anti-Smad6 polyclonal antibody, anti-oligodendrocytic basic helix–loop–helix transcription factor 2 (OLIG2) polyclonal antibody, DyLight^{TM}547-conjugated antibody to rabbit IgG heavy and light chains, Cy2-conjugated antibody to mouse IgG_1 and AP-conjugated antibody to rabbit IgG heavy and light chains were obtained from Signal Antibody Technology Inc. (Silicon Valley, CA), CALBIOCHEM (Darmstadt, Germany), BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA), Upstate Biotechnology (Lake Placid, NY), IMGENEX (San Diego, CA), IBL (Takasaki, Japan), Pierce Biotechnology Inc. (Rockford, IL), CHEMICON International Inc. (Temecula, CA) and Leinco Technologies Inc. (Ballwin, MO), respectively.

2.2. Cell culture

SFME cells, a gift from Dr. S. Shirahata (Kyushu University, Fukuoka, Japan), were cultured in a humidified 7% CO_2–93% air atmosphere at 37 °C in 60 mm-diameter dishes pre-coated with 10 μg/ml fibronectin. The basal nutrient culture medium was a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 (Ham and McKeehan, 1979; Mather and Sato, 1979) containing 15 mM HEPES, pH 7.4, 1.2 g/l sodium bicarbonate, 10 nM sodium selenite and 10 μg/ml gentamicin, supplemented with insulin (10 μg/ml), transferrin (25 μg/ml) and EGF (50 ng/ml). Cell passages were accomplished by rapid trypsinization with 0.2% crude trypsin and 1 mM EDTA in PBS without calcium or magnesium, followed by dilution in the culture medium at room temperature. The medium containing the collected cells was centrifuged at $250 \times g$ at 4 °C for 7 min and the supernatant was removed. The cells were suspended in the culture medium without the supplements, plated at 1×10^5 cells/dish and cultured again in the medium containing the supplements.

2.3. Cell proliferation assays

SFME cells cultured continuously in the serum-free medium were detached from stock dishes (stock SFME cul-

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