

Letter

## A new insight into proliferative action of bisphenol A at low-dose

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**ABSTRACT** — There are many subclones of human breast cancer MCF-7 cells that respond to different degrees of estrogen and that have been used for testing the estrogenic activity of environmental chemicals such as bisphenol A. Here, we examined the estrogenicity of bisphenol A in an MCF-7 subclone. It was not able to detect the increment of BrdU incorporation by neither  $10^{-7}$  M bisphenol A alone nor 10 ng/mL epidermal growth factor (EGF) alone in the cells ( $1 \times 10^4$  cells) up to 50 hr. However, in the presence of 10 ng/mL EGF, bisphenol A dramatically increased cell proliferation ( $ED_{50} = 10$  pM). The synergistic responses were the chemical concentration-dependent manner. Thus, in this study, we demonstrate the new insight into the action of bisphenol A in the MCF-7 subclonal cell.

**Key words:** Bisphenol A, EGF, Proliferation, MCF-7 cells

### INTRODUCTION

Endocrine-disrupting chemicals such as bisphenol A have been received considerable public concerns, particularly because of a large family of estrogenic nature of the chemicals (Howdeshell *et al.*, 1999). Furthermore, there has been discrepancy about low-dose effects of bisphenol A on MCF-7 human breast carcinoma cells and laboratory animals (Welshons *et al.*, 2003). One claimed that discrepancy of the data among investigators about low-dose effects of bisphenol A could come from the contamination of estrogenic substances from materials used for bioassay (Welshons *et al.*, 2003). Other possibility might be the level of endogenous estradiol that might substantially increase the sensitivity of the target organs to estrogenic chemicals (Howdeshell *et al.*, 1999; Welshons *et al.*, 2003). The molecular evidence, therefore, has been needed to elucidate the potential effects of low-dose of bisphenol A.

Wildspread use of MCF-7 cells as a model system for breast cancer has led to variations in these cells between different laboratories. Although several reports have addressed these differences in terms of proliferation and estrogenic activity, variations in sensitivity to environmental chemicals have yet been limited to be described (Lippman *et al.*, 1980; Villalobos *et al.*, 1995).

During the course of the study of estrogen responsiveness using the estrogen receptor alpha-positive MCF-7 cells cultured in phenol red-free medium and 10% charcoal-stripped fetal bovine serum (FBS), we found that cell proliferation by estradiol of MCF-7 cells was extremely slow and that supplementation of growth factors such as epidermal growth factor (EGF) facilitated estradiol-induced cell growth (Ishido *et al.*, 2001). By the analogy of these observations, we here examined the effects of EGF on bisphenol A-elicited cell proliferation of MCF-7 cells.

### MATERIALS AND METHODS

#### Materials

Bisphenol A was purchased from Wako Chemicals Corp. (Tokyo, Japan). Epidermal growth factor (EGF) was from Sigma Chemicals Co. (St. Louis, MO, USA).

#### Cell culture

MCF-7 cells were kindly provided by Dr. Liburdy (UCLA, Berkley, USA) and grown in Dulbecco's modified Eagle's Medium (Invitrogen Co., Carlsbad, CA, USA) supplemented with 10% FBS (Invitrogen), penicillin (100 U/mL), and streptomycin (100  $\mu$ g/mL) in a humidified atmosphere of 95% air: 5% CO<sub>2</sub> at 37°C. For examination of effects of estrogenic activity, the cells were cul-

tured in phenol-red-free medium (Sigma Chemicals) and supplemented with 10% dextran-coated charcoal-stripped FBS. The cells were subcultured (1:4) 1 to 2 times per week.

### Bromodeoxyuridine (BrdU) incorporation

DNA synthesis was determined via BrdU incorporation as previously described (Ishido *et al.*, 1999, 2001). MCF-7 cells were grown in a 96-well plate (Coaster Co., Cambridge, MA, USA). The cells were treated with test additives for indicated periods at 37°C. Then, 10  $\mu$ M BrdU was added for a further 3 hr at 37°C. The cells were fixed with 70% ethanol in 0.5 M HCl for 30 min at -20°C. Following treatment with nuclease, the cells were incubated with anti-BrdU antibody conjugated to peroxidase (Roche Applied Science, Mannheim, Germany). Bound enzymes were detected with the substrates ABTS®(2,2'-azino-di-[3-ethylbenzthiazolinesulfonate]) (Roche Applied Science) and quantified by measuring absorbance at 405 nm with an enzyme-linked immunosorbent

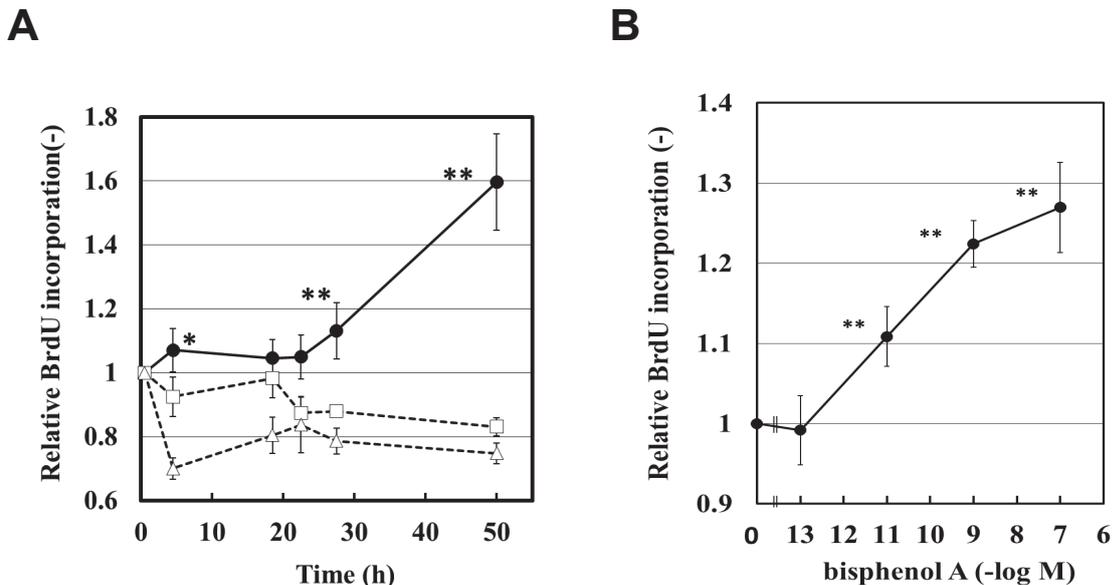
assay (ELISA) plate reader (BIO-TEK Instruments, Inc., Winooski, VT, USA; model  $\mu$ Quant MQX200).

### Statistics

Statistical analyses were carried out via Student's t-test using the StatView Ver. 5.0 statistical software package (SAS Institute Inc., Cary, NC, USA).

## RESULTS AND DISCUSSION

First, the growth stimulatory effects of bisphenol A on the MCF-7 cells were evaluated by bromodeoxyuridine (BrdU) incorporation. The MCF-7 cells were replaced to phenol red-free medium supplemented with 10% dextran-coated charcoal-stripped FBS 24 hr before assay. The cells were incubated with bisphenol A, EGF, or the combination of both for indicated periods and labeled with BrdU. Figure 1A shows that neither 10<sup>-7</sup> M bisphenol A nor 10 ng/mL EGF promoted cell growth at least up to 50 hr. However, in the presence of 10 ng/mL EGF, 10<sup>-7</sup>



**Fig. 1.** A, Quantification of relative BrdU incorporation into MCF-7 cells by ELISA. The MCF-7 cells ( $2 \times 10^4$  cells) were replaced into phenol red-free medium and 10% charcoal-stripped FBS 24 hr before assay. Then, bisphenol A ( $10^{-7}$  M; -□-), EGF (10 ng/ml; -○-), or the combination of both (-●-) were incubated for indicated periods. Following incubation with 10  $\mu$ M BrdU for another 3 hr, the incorporated BrdU was quantified by ELISA. The data are mean  $\pm$  S.E. ( $n = 4$ ). Asterisks denote significant differences between bisphenol A-treated cells and the combined additives-treated cells ( $p < 0.05$ ). Double asterisks denote significant differences between EGF-treated cells and the combined additives-treated cells ( $p < 0.05$ ). B, Dose-response curve of cell proliferation by bisphenol A of MCF-7 cells. The MCF-7 cells ( $2 \times 10^4$  cells) were replaced into phenol red-free medium and 10% charcoal-stripped FBS 24 hr before assay. Then, various concentration of bisphenol A as indicated was incubated for 36 hr in the presence of 10 ng/mL EGF. Following incubation with 10  $\mu$ M BrdU for another 3 hr, the incorporated BrdU was quantified by ELISA. The data are mean  $\pm$  S.E. ( $n = 3$ ). Double asterisks denote a significant increment by bisphenol A ( $p < 0.05$ ).

## Sensitization by EGF of BPA action

M of bisphenol A dramatically facilitated BrdU incorporation. It was about 1.6 fold stimulation for 50 hr.

Figure 1B shows that the synergistic responses were dependent on the chemical concentration. Even in the presence of 10 ng/mL of EGF,  $10^{-13}$  M of bisphenol A failed to stimulate the cell growth, whereas  $10^{-11}$  M of bisphenol A significantly facilitated DNA synthesis.

Cross-talk signaling between the estrogen receptor and the EGF receptor has been observed in reproductive organs (Levin, 2003; Smith, 1998). In this study, we show the new mechanism of acceleration of proliferative activity of bisphenol A at low-dose by synergism with EGF; estrogenic action of bisphenol A could be coupled to the growth factor signaling, most likely through activation of estrogen receptor- $\alpha$  (Ishido *et al.*, 2001). The molecular mechanism of the estrogenic action of bisphenol A has not been fully understood. The elucidation of a cross-talk mechanism between EGF and estrogen receptor activation by bisphenol A would explain the discrepancy between synergistic growth effects by bisphenol A and binding efficacy of bisphenol A to estrogen receptor.

Our data consequently require new assumption for risk assessment of such chemicals; it should be based on not only toxicological concentration of the chemical but also chemical concentration for synergistic activation.

**Conflict of interest----** The authors declare that there is no conflict of interest.

## REFERENCES

- Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenberg J.G. and Vom Saal, F.S. (1999): Environmental toxins: Exposure to bisphenol A advances puberty. *Nature*, **401**, 763-764.
- Ishido, M., Suzuki, T., Adachi, T. and Kunimoto, M. (1999): Zinc stimulates DNA synthesis during its antiapoptotic action, independently with increments of an antiapoptotic protein, Bcl-2, in porcine kidney LLC-PK<sub>1</sub> cells. *J. Pharmacol. Exp. Ther.*, **290**, 923-928.
- Ishido, M., Nitta, H. and Kabuto, M. (2001): Magnetic fields (MF) of 50 Hz at 1.2 microT as well as 100 microT cause uncoupling of inhibitory pathways of adenylyl cyclase mediated by melatonin 1a receptor in MF-sensitive MCF-7 cells. *Carcinogenesis*, **22**, 1043-1048.
- Levin, E.R. (2003): Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. *Mol. Endocrinol.*, **17**, 309-317.
- Lippman, M.E., Srobl, J. and Allerga, J.C. (1980): Effects of hormones on human breast cancer cells in tissue culture. (C.M. McGrath, M.J. Brennan, M.A. Rich, eds.) In *Cell Biology of Breast Cancer*, pp.205-276, Academic Press, New York.
- Smith, C.L. (1998) Cross-talk between peptide growth factor and estrogen receptor signaling pathways. *Biol. Reprod.*, **58**, 627-632.
- Villalobos, M., Olea, N., Brotons, J.A., Olea-Serrano, M.F., Ruiz deAlmodovar, J.M. and Pedraza, V. (1995): The E-screen assay: a comparison of different MCF-7 cell stocks. *Environ. Health Perspect.*, **103**, 844-850.
- Welshons, W.V., Thayer, K.A., Judy, B.M., Taylor, J.A., Curran, E.M. and Vom Saal, F.S. (2003): Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ. Health Perspect.*, **111**, 994-1006.