BISPHENOL A INDUCES BREAST CANCER CELL APOPTOSIS

Shunichiro Kubota, Sachiko Obara, Taeko Miyauchi and Takahiro Nemoto

Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Introduction

Considerable attention has been focused on environmental chemicals which disrupt various tissues via steroid receptor. Bisphenol A, 2, 2-bis (4-hydroxyphenyl) propane, is a major component of epoxy resins and exhibits estrogenic activities[1]. The chemical structure of bisphenol A resembles that of diethylstilbestrol which causes carcinogenesis[2, 3]. Environmental chemicals with estrogenic activity are considered to cause reproductive disorders, endocrine disorders, variety of cancers such as prostate, ovarian and breast cancer[4].

Although much information is available concerning growth promoting effect of bisphenol A[2], little is known about the effect of bisphenol A on cell death and its mechanism[5, 6]. Much is not known whether estrogen receptor is involved in bisphenol A-induced cell death.

We undertook this study to elucidate whether bisphenol A induced apoptosis of breast cancer cell lines, estrogen receptor-positive MCF-7 and -negative MDA-231 and whether it stimulated caspase-1, -3, -6, -8 and -9 activities in both cells.

Materials and Methods

Reagents. Bisphenol A was purchased from Kanto Chemicals Co., Ltd. (Tokyo, Japan). Cell culture media were obtained from Sigma (St. Louis, MO).

Cells. Human breast cancer cell lines (MCF-7 and MDA-231) were obtained from Japanese Cancer Research Resources Bank (Osaka) and grown in Dulbecco's Modified Eagles' Media (DMEM) without phenol red containing 10% fetal bovine serum (JRH, Lenexa, KS). Bisphenol A (0.01-100 μM) was added to subconfluent cells in 6 ml serum-free DMEM media (100 mm dishes) and incubated for 1 to 24 hours. Cells were harvested for DNA preparation and caspase assay. The cell extracts were prepared using lysis buffer (10 mM Tris-HCl buffer, pH7.5, containing 1mM EDTA, 0.5 μg/ml aprotinin, 1 μg/ml leupeptin, and 0.2 mM PMSF) and centrifuged at 12,000 rpm for 10 min at 4 °C. Protein concentration of the supernatant was measured using a Bradford method.

Caspase assay. Caspase1, 3, 6, 8 and 9 activities were measured fluorometrically using Ac-YVAD-MCA, Ac-DEVD-MCA, Ac-VEID-MCA, Ac-IETD-AMC and Ac-LEHD-MCA as fluorogenic substrates, respectively. Twenty μg of cell extracts was used and the activities of caspases were expressed as %, compared to the activity at 0 h (100%).

Results and Discussion

The effects of bisphenol A on cell death were investigated in vitro using human breast cancer cell lines, MCF-7 and MDA-231. Preliminary experiment showed that lower doses of bisphenol A (0.01-1 μM) didn't significantly affect cell viability of both cell lines, but higher dose (100 μM) of

bisphenol A induced cell death in a time-dependent manner (1-24 h). Therefore, we used 100 μM bisphenol A in the present experiment. To examine whether bisphenol A induced cell apoptosis, we first analyzed the effect of bisphenol A (100 μM) on DNA ladder formation. Bisphenol A induced DNA ladder formation in both cells (figure not shown), estrogen receptor-positive MCF-7 and –negative MDA-231 cells. The data suggest that bisphenol A induced apoptosis of both cells independent of estrogen receptor. Time course study (0, 1, 3, 5, 7, 9, 11 and 24 h) of the effects of bisphenol A (100 μM) on the induction of caspases was performed. Bisphenol A did not significantly affect the activities of caspases 1, 8 and 9 in both MCF-7 and MDA-231 cells (not shown). In contrast, as shown in figures 1 and 2, bisphenol A significantly induced caspase 3 activities in a time-dependent manner in MCF-7 (1-11 h) and in MDA-231 cells (1-7 h), respectively. As shown in figures 3 and 4, bisphenol A also induced caspase 6 activities in a time-dependent manner in MCF-7 (1-24 h) and in MDA-231 cells (3-7 h), respectively. The magnitude of induction of caspases 3 and 6 was small although it was statistically significant. Therefore, we cannot rule out the possibility that other caspases except caspases 1, 8 and 9 were involved in bisphenol A-induced apoptosis.

Although it is well-known that lower dose of bisphenol A stimulates human mammary carcinoma cell growth (MCF-7) via estrogen receptor pathway[2], little is known about the effect of bisphenol A on cell death and its mechanism. The present study is the first demonstration that bisphenol A induced human mammary carcinoma cell apoptosis independent of estrogen receptor via caspases 3 and 6 signaling pathway.

References

1. Reid E.E. and Wilson E. (1944) J Amer Chem Soc 66, 967.
2. Krishan A., Stathis P., Permuth S., Tokes I. and Feldman D. (1993) Endocrinology 132, 2278.
3. Roy D., Palangat M., Chen C-W., Thomas R.D., Colerangle J., Atkinson A. and Yan Z-J. (1997) J Toxi Environ Health 50, 1.
4. Stenmetz R., Mitchner N., Grant A., Allen D., Bigsby R. and Ben-Jonathan N. (1997) Endocrinology 139, 2741.
5. Hughes PJ, McLellan H, Lowes DA, Kahn SZ, Bilmen JG, Tovey SC, Godfrey RE, Michell RH, Kirk CJ and Michelangeli F. (2000) Biochem Biophys Res Commun 277, 568.
6. Nakagawa Y. and Tayama S. (2000) Arch Toxicol 74, 99.

Acknowledgement

This study was supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health and Welfare of Japan.

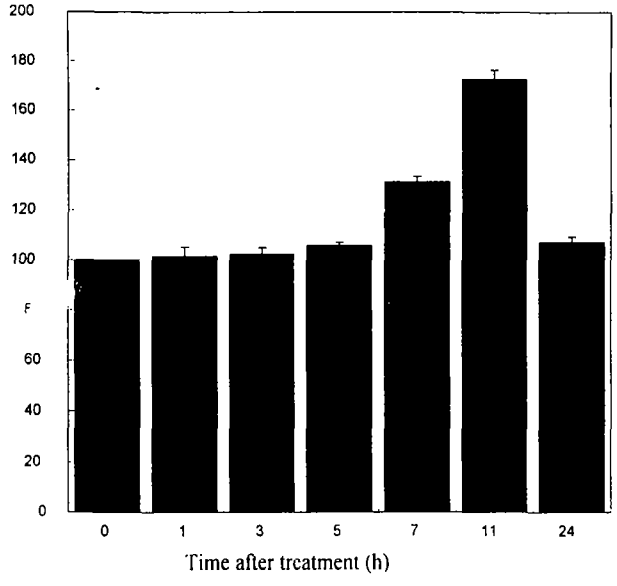

Fig. 1 Time course of caspase 3 in MCF-7

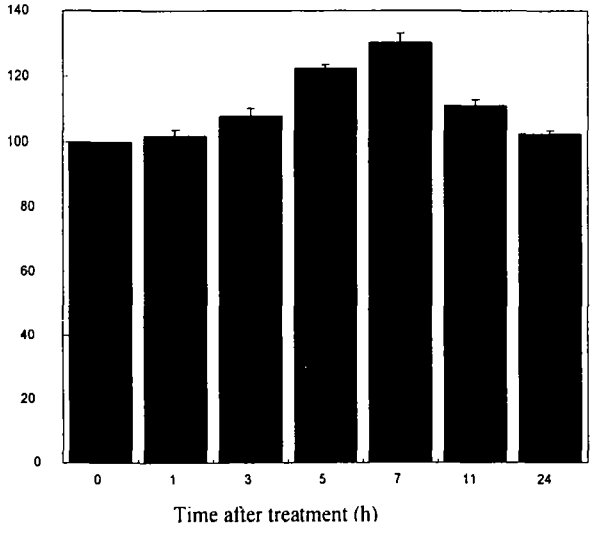

Fig. 2 Time course of caspase 3 in MDA-231

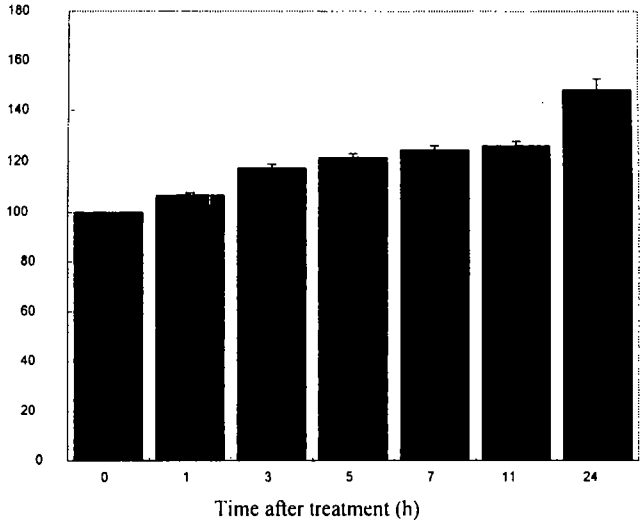

Fig. 3 Time course of caspase 6 in MCF-7

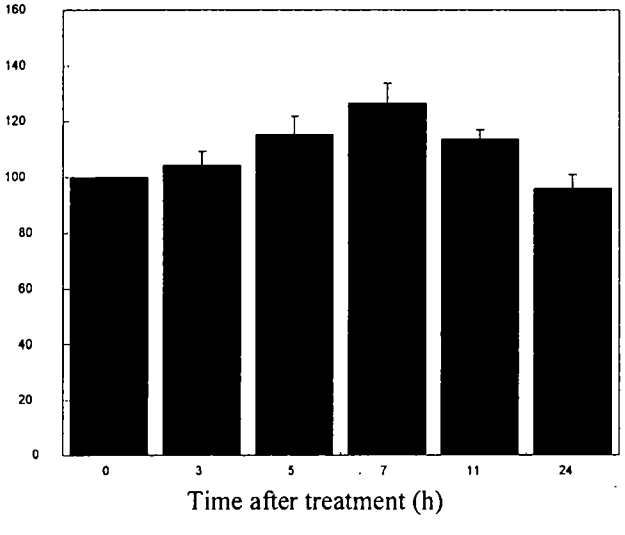

Fig. 4 Time course of caspase 6 in MDA-231