BISPHENOL A SUPPRESSES MMP-9 SECRETION BY MDA CELLS

Shunichiro Kubota and Yousuke Seyama

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, a diphenylcompound containing 2 hydroxyl groups in para positions, is a major component of epoxy resins. The estrogenic activities ofbisphenol A was reported previously (1). The chemical structure of bisphenol A resembles that of DES which causes carcinogenesis (2). Recent reports showed that bisphenol A induces cellular transformation (3) and suppresses cytochrome p450 expression (4).

Tumor metastatic process and wound healing process containing matrix remodeling involve cell attachment to extracellular matrix and basement membrane and degradation of them (5,6). During the process of degrading extracellular matrix proteins and basement membrane, matrixmetalloproteinases (MMPs) play important roles. MMPs are classified into, at least, three types according to their substrate specificity, interstitial collagenase (MMP-1 and MMP-8), stromelysin (MMP-3 and MMP-10), and typeIV collagenase (MMP-2 and MMP-9 also called 72kDa gelatinase and 92kDa gelatinase, respectively)(6). The effect of bisphenol A on MMP secretion by culture cells in vitro has not been reported yet. We undertook this study to elucidate the effect of bisphenol A on secretion of MMPs by culture cells in vitro.

Materials and Methods

Reagents. Bisphenol A was purchased from Kanto Chemicals Co., Ltd. (Tokyo, Japan). Cell culture media and gelatin were obtained from Nikken Bio Medical Laboratory (Tokyo, Japan) and Sigma (St. Louis, MO, USA), respectively.

Cells. Human breast cancer cells (MDA) and human leukemia cells (U937) were obtained from Japanese Cancer Research Resources Bank (JCRRB)(Tokyo), and grown in Dulbecco's Modified Eagles' Media (DMEM) containing 10% fetal bovine serum. and in PRMI media containing 10% fetal bovine serum. 5x105 cells in 1ml DMEM or in PRMI media were incubated for 6 and 24 hours. The number of the cells was not significantly increased after 24 hours by counting cells using *Burger-Turk* hemocytometer. The serum free conditioned medium(1 ml) was harvested for zymogram analysis.

Zymogram. 45μl of conditioned medium collected from MDA cells, and U937 was used for zymogram analysis as described previously (7). The activityof MMPs (MMP-2 and MMP-9) was quantified using Photoshop and Histogram analysis program in Macintosh.

Results and Discussion

The effects of bisphenol A on MMP-9 secretion were investigated using the zymogram analysis. Incubation times with bisphenol A were 6 hr and 24 hr. U937 cells secrete MMP-9. As shown in Figure 1 bisphenol A (0.001uM, 0.01 uM, 0.1 uM, 1 uM, 10 uM and 100 uM) did not significantly affect the secretion of MMP-9 by U937 cells.

We next investigated the effects of bisphenol A (1 uM, 10 uM, 50 uM, and 100 uM) on MMP-2 and MMP-9 secreted by MDA cells. MDA cells predominantly secrete MMP-9. Incubation times with bisphenol A were 6 hr and 24 hr. As shown in Figure 2, at6 hr incubation time bisphenol A concentrations at 1 uM, and 10 uM did not significantly affect the secretion of MMP-9. On the contrary, bisphenol A concentrations at 50 uM, and 100 uM significantly suppressed MMP-9 secretion. At 24 hr incubation time bisphenol A concentrations at 100 uM significantly suppressed MMP-9 secretion, but 1 uM, 10 uM, and 50 uM bisphenol A did not significantly affect the secretion of MMP-9. Concerning MMP-2 secretion the amount of secreted MMP-2 was not sufficient to evaluate the effect of bisphenol A.

To our knowledge this is the first report that bisphenol A affects the secretion of MMP-9 by human mammary carcinoma cells in vitro. MMP-9 is considered to be involved in physiological state such as development, and also in pathological state such as tumor metastatic process and wound healing. This study is ongoing, and we plan to elucidate whether estrogen receptor is involved in this phenomenon using tamoxifen. We also investigate whether bisphenol A affects the secretion of MMPs by normal cells such as fibroblasts and endothelial cells.

References

1 . Reid EE, and Wilson E (1944) J Amer Chem Soc 66:967-969.
2. IRAC, IARC monographs on the evaluation of the carcinogenic risk of chemicals. Sex hormones (II), Vol 21 International Agency for Research on Cancer, Lyon (1979).
3. Tsutsui T, Tamura Y, Yagi E, Hasegawa K, Takahashi M, Maizumi N, Yamaguchi F and Barrett JC (1998) Int J Cancer 75:290-294.
4. Hanioka N, Jinno H, Nishimura T, and Ando M (1998) Arch Toxicol. 72: 387-394.
5. Liotta LA (1986) Cancer Res 46: 1-7.
6. Matrisian LM (1990) TIG 6: 121-125.
7. Kubota s, Fridman R, and Yamada Y. (1991) Biochim. Biophys. Res. Commun 176: 129-136.

Fig. 1 Effect of Bisphenol A on MMP-9 Secretion by U937 cells

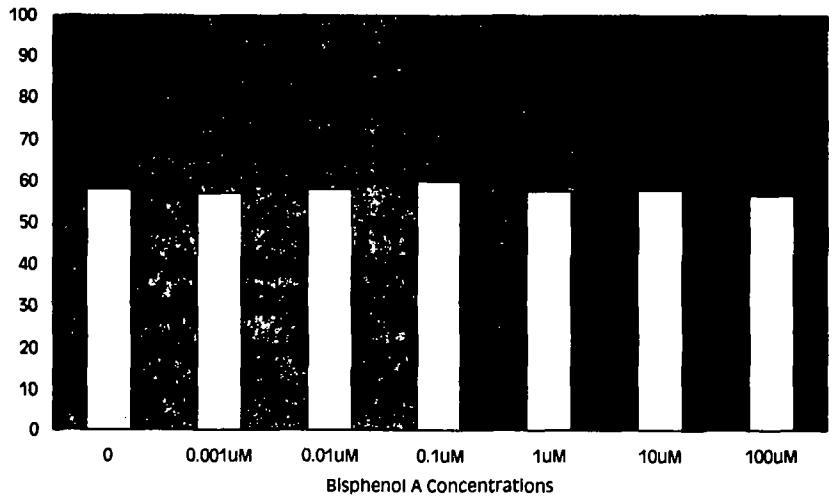

Fig. 2 Effect of Bisphenol A on MMP-9 Activities by MDA Cells