DIFFERENTIAL EFFECTS OF PARA-NONYLPHENOL ON PROTEINASE SECRETION BY HUMAN LEUKEMIA CELLS

Shunichiro Kubota , Sachiko Ohara, and Taeko Miyauchi

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

Environmental chemicals disrupt endocrine functions of various organs, including the reproductive systems in humans (1-3). Chemicals mimic steroid hormones through interactions with the estrogen receptor. Numerous environmental chemicals which can bind to the estrogen receptor and induce expression of the estrogen receptor-mediated genes have been identified (4, 5). Alkylphenols which are widely used as surfactants in plastics are degraded to para-nonylphenol. Para-nonylphenol binds to the estrogen receptor and induces estrogen-dependent gene expression (4). Human peripheral mononuclear cells express the estrogen receptor (6, 7).

The interaction between cells and the extracellular matrix is critical for normal development, wound healing, inflammation, and cancer metastasis. Modulation of cell-extracellular matrix interactions occurs through hydrolysis of the extracellular matrix by matrix metalloproteinases (8, 9). Metalloproteinases play a pivotal role in regulation of angiogenesis, cell proliferation, differentiation, and cell death. Uncontrolled proteolysis due to up-regulation or down-regulation of metalloproteinases contributes to abnormal development and to the generation of pathological conditions such as inflammation and cancer metastasis. Metalloproteinases are enzymes that regulate cell-extracellular matrix interactions, and these enzymes are classified into four types according to their substrate specificity, collagenases, stromelysins, elastases, and gelatinases (72kDa and 92kDa gelatinases)(9). 92 kDa gelatinase is utilized by myeloid and lymphoid cells for migration across basement membranes (10, 11). Effects of para-nonylphenol on 92 kDa gelatinase and casein-degrading proteinase secretion by cells including peripheral lymphoctes and U937 cells in vitro have not been documented. We undertook this study to elucidate effects of para-nonylphenol on secretion of 92kDa gelatinases and casein-degrading proteinases by human leulemia cells in vitro.

Materials and Methods

Materials. Cell culture media (RPMI 1640) with or without phenol red were obtained from Sigma (Tokyo, Japan), and Gibco BRL (Tokyo, Japan), respectively. Para-nonylphenol, obtained from Kanto Chemicals Co., Ltd. (Tokyo, Japan) was diluted with dimethyl sulfoxide or RPMI 1640 media to a final concentration.Gelatin,17ß estradiol, alpha-casein and trypan blue solution were obtained from Sigma (St. Louis, MO, USA). ICI 182780 was purchased from Tocris (Ballwin, MO, USA).

Cells. U937 cells were kindly provided by Dr. K. Kano (The University of Tokyo), and grown in RPMI 1640 media containing 10% fetal bovine serum. 10^6 cells in 1ml RPMI 1640 media in 24 well plates were incubated for 24 h. Dimethyl sulfoxide (0.2%) was added to culture media, as a

control. The serum free conditioned medium (1ml) was harvested for zymography.

Zymography. Forty-five µl out of 1 ml serum free conditioned medium was used for gelatin or alpha-casein substrate zymography as described (12). Quantification of proteinase activity was done by scanning gels densitometrically. The number of pixels was counted using Macintosh Adobe Photoshop 5.0 J and an Histogram analysis program (12). Statistical significance was assessed using StatView 4.5.

Treatment cells with para-nonylphenol. At 24 h incubation after adding para-nonylphenol, or para-nonylphenol with tamoxifen, or para-nonylphenol with ICI 182780, or tamoxifen, or ICI 182780 in serum free RPMI 1640 media, cells were harvested, and centrifuged at 1,500 rpm for 10 min. Cell pellets were used for cell counting. Viability of the cells was judged using the trypan blue exclusion method. Cells were mixed with 0.4% trypan blue 1:1 and viable cells were counted using a hemocytometer.

Results and Discussion

Effect of para-nonylphenol on 92 kDa gelatinase secretion

We investigated effects of para-nonylphenol on gelatinase secretion by human promyelocytic leukemia U937 cells. U937 cells predominantly secrete 92kDa gelatinase. As shown in Figure 1 (upper panel), at 24 h incubation time para-nonylphenol (0.05, 0.5, 5, 25, 50, 100, 200, and 300 µM) dose-dependently suppressed the secretion of 92 kDa gelatinase. Results of the quantification are shown in Figure 1 (lower panel). Para-nonylphenol concentrations at 50, 100, 200, and 300 µM suppressed 92 kDa gelatinase secretion 45.0 ± 5.2 % ($p<0.05$), 66.0 ± 4.9 % ($p<0.05$), 84.5 ± 6.2 % ($p<0.05$), and 84.0 0 ± 5.0 % ($p<0.05$), respectively, compared to control. Tamoxifen (0.5 µM) completely blocked the suppressive effects of 50 µM para-nonylphenol, but not the effects of 100, 200 and 300 µM para-nonylphenol. Therefore, we next determined if a higher concentration (4.0 µM) of tamoxifen would block the suppressive effects of para-nonylphenol (100, 200, and 300 µM), but it did not do so (Figure 1). We also determined if ICI 182780 (10 µM), an anti-estrogen would block the suppressive effects of para-nonylphenol (100, 200, and 300 µM), but it did not (Figure 1).

Effect of estradiol on 92 kDa gelatinase secretion

We next studied the effects of 17 ß estradiol on 92 kDa gelatinase secretion for 24h by U 937 cells. We used physiological and supraphysiological concentrations of 17 ß estradiol (5 nM, 50 nM, 1 µM, 2.5 µM, 5 µM, 50 µM, 100 µM, and 200 µM). 17 ß estradiol did not significantly suppress 92 kDa gelatinase secretion by U937 cells (not shown). We also studied effects of 17 ß estradiol on 92 kDa gelatinase secretion for 48h by U 937 cells, but it did not significantly suppress 92 kDa gelatinase secretion (not shown).

Effect of para-nonylphenol on casein-degrading proteinase secretion

We further studied the effect of para-nonylphenol on casein-degrading proteinase secretion by U937 cells. U937 cells secrete small amount of several casein-degrading proteinases (MW; between 40-80 kDa). Para-nonylphenol strongly induced these proteinase secretion by U937 cells in a dose-dependent manner.

In the present study we have found that para-nonylphenol differentially affects the secretion of different proteinases by U937 cells. To our knowledge this is the first report that para-nonylphenol modulates different proteinase secretion by human cells differentially.

The suppressive effect of 50 μM para-nonylphenol was completely resuced by tamoxifen (0.5 μM) in U937 cells. The result suggests that the suppressive effect of para-nonylphenol was mediated via the estrogen receptor. However, the suppressive effect of higher doses (100, 200, and 300 μM) of para-nonylphenol in U937 cells was not blocked by tamoxifen (4 μM). Therefore, we tested another anti-estrogen, ICI 182780 whether it would block the suppressive effects of higher doses (100, 200, and 300 μM) of para-nonylphenol, but it did not. There are at least two possibilities;one is that the suppressive effect of higher doses (100, 200, and 300 μM) of para-nonylphenol was not mediated via estrogen receptor. The other one is that the dose of para-nonylphenol used was too high to cause a suppression. Since a higher concentration of tamoxifen (>4 μM) and ICI 182780 (>10 μM) suppressed 92 kDa gelatinase secretion by U937 cells, it was not feasible to test whether a higher concentration of tamoxifen and ICI 182780 would block the suppressive effects of para-nonylphenol (100, 200, and 300 μM). Physiological and supraphysiological concentrations of 17 ß estradiol did not significantly suppress 92 kDa gelatinase secretion by lymphocytes and U 937 cells. The results suggest that with regard to 92 kDa gelatinase regulation, para-nonylphenol interacts with the estrogen receptor and transduces signals in a manner distinct from that of estradiol. The different action from estradiol at the estrogen receptor was reported in case of bisphenol, an estrogenic chemical (14). Down-stream signal transduction after the estrogen receptor remains to be elucidated.The results suggest that with regard to 92 kDa gelatinase regulation, para-nonylphenol interacts with the estrogen receptor and transduces signals in a manner distinct from that of estradiol. The different action from estradiol at the estrogen receptor was reported in case of bisphenol, an estrogenic chemical (14). Down-stream signal transduction after the estrogen receptor remains to be elucidated. Concerning casein-degrading proteinases we are currently studying to identify them by using antibodies against various proteinases.

References

1. Colborn, T. (1995) Environmental oestrogen: health implications for humans and wildlife. *Environ. Health Perspective* 103, 135.
2. Roy, D., Palangat, M., Chen, C.W., Thomas, R.D., Colerangle, J., Atkinson, A., and Yan, Z.J. (1997) Biochemical and molecular changes at the cellular level in response to exposure to environmental estrogen-like chemicals. *J. Toxicol. Environ. Health* 50, 1.
3. Safe, S.H. (1995) Do environmental estrogens play a role in development of breast cancer in women and male reproductive problems ? *Human and Ecological Risk Assessment*, 1,17.
4. Bolger, R., Wiese, T.E., Ervin, K, Nestich, S., and Checovich, W. (1998) Rapid screening of environmental chemicals for estrogen receptor binding capacity. *Environ Health Perspect.* 106, 551.
5. Danzo, B.J. (1997) Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. *Environ. Health Perspect.* 105, 294.
6. Danel, L., Souweine, G., Monier, J.C., and Saez, S. (1983) Specific estrogen binding sites in human lymphoid cells and thymic cells. *J. Steroid Biochem.* 18, 559.

7. Suenaga, R., Evans, M.J., Mitamura, K., Rider, V., and Abdou, N.I (1998)Peripheral blood T cells and monocytes and B cell lines derived from patients with lupus express estrogen receptor transcripts similar to those of normal cells. *J. Rheumatol.* 25, 1305.
8. Massova, I., Kotra, L.P., Fridman, R., and Mobashery, S. (1998) Matrix metalloproteinases: structures, evolution, and diversification. *FASEB J.* 12, 1075.
9. Nelson, A.R., Fingleton, B., Rothenberg, M.L., and Matrisian, L.M. (2000) Matrix Metalloproteinases: Biologic Activity and Clinical Implications. J. Clin. Oncology, 18, 1135.
10. Hayashi, M., Schellenberg, R.R., Tsang S., Roberts, C.R. (1999) Matrix metalloproteinase-9 in myeloid cells: implications for allergic inflammation. *Int. Arch. Allergy Immunol.* 118, 429.
11. Lemjabbar H, Gosset P, Lamblin C, Tillie I, Hartmann D, Wallaert B, Tonnel AB, Lafuma C (1999) Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. *Am. J. Respir. Cell Mol. Biol.* 159, 1298.
12. Kubota,S., Takeda, K., Yamada, T., Nomura, Y., Takeda,M., Iwamoto, S., & Seyama,Y. (1996) Tumor Necrosis Factor Alpha- and lymphotoxin stimulate human myeloblastic leukemia cell (ML-1) invasion through a reconstituted basement membrane (matrigel) with concomitant induction of 92kDa gelatinase secretion. *Cancer Lett.*, 98, 233.
13. Nishiguchi, T., Kobayashi, T., Sugimura, M., and Terao, T. (1999) Upregulation of thrombomodulin antigen levels in U937 cells by combined stimulation with estradiol-17beta and vitamin K2. Semin. Thromb. Hemost., 25, 509.
14. Gould, J.C., Leonard, L.S., Maness, S.C, Wagner, B.L., Conner, K., Zacharewski, T., Safe S, McDonnell, DP., and Gaido, K.W. (1998) Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Mol. Cell Endocrinol.* 142, 203.

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Figure 1 Effects of para-nonylphenol on 92 kDa gelatinase secretion by U937cells

Effects of para-nonylphenol (0.05, 0.5, 5, 25, 50, 100, 200, and 300 μM) on 92kDa gelatinase secretion by U937 cells were analyzed using gelatin zymography. Serum free conditioned media were collected 24 h after the addition of para-nonylphenol. A typical zymography is shown in the upper panel. The experiments were done three times, and quantitative data are shown in lower panel. Quantification was done as described in Methods, and values, expressed as percentages compared to control (100%), are means ± SD of three experiments (lower panel). Lane or column 1, 0.2 % DMSO (control); lane or column 2, RPMI 1640 media alone; lanes or columns 3-10, para-nonylphenol 0.05, 0.5, 5, 25, 50, 100, 200, and 300 μM, respectively; lane or column 11, tamoxifen 0.5 μM; lane or column 12, para-nonylphenol 50 μM + tamoxifen 0.5 μM; lanes or columns 13 and 14, tamoxifen 3 and 4 μM, respectively; lanes or columns 15-18, tamoxifen 4 μM + para-nonylphenol 50, 100, 200 and 300 μM, respectively; lanes or columns 19 and 20, ICI 182780 1 and 10 μM, respectively; lanes or columns 21-23, ICI 182780 10 μM + para-nonylphenol 100, 200 and 300 μM, respectively.