EFFECTS OF PARA-NONYLPHENOL ON MATRIX METALLOPROTEINASE SECRETION BY HUMAN LEUKEMIA CELLS

Shunichiro Kubota

Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Tokyo 153-8902, Japan

Introduction

Environmental chemicals disturb endocrine functions of various organs in humans[1-3]. Chemicals mimic steroid hormones through interactions with the estrogen receptor. A number of environmental chemicals which can bind to the estrogen receptor induce expression of various genes[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[5]. Human peripheral mononuclear cells and leukemia cells express the estrogen receptor[6,7].

The turnover of the extracellular matrix is critical for normal development, wound healing, inflammation, and cancer metastasis. The extracellular matrix is hydrolyzed by matrix metalloproteinases (MMP)[8,9]. MMPs play pivotal roles in regulation of angiogenesis, cell proliferation, differentiation, and cell death. Abnormal proteolysis due to up-regulation or down-regulation of MMPs may lead to abnormal angiogenesis, cell proliferation, differentiation, and cell death. MMPs are classified into four types according to their substrate specificity, collagenases, stromelysins, elastases, and gelatinases[9]. Collagenases exist as three distinct molecules, the fibroblast type (MMP-1, collagenase-1)[10], the neutrophil type (MMP-8) [11], and collagenase-3 (MMP-13)[12]. They all degrade type I, II, and III collagens as well as type VII and X collagens, and alpha-casein. MMP-8 effectively degrade type I and II collagens, and are stored in granules of human peripheral blood PMN[13]. In the present study the author studied the effect of para-nonylphenol on secretion of alpha-casein-degrading MMPs by human leukemia Jurkat cells in vitro.

Materials and Methods

Cell culture media (RPMI 1640), alpha-casein, anti-MMP-8 antibody and doxycycline were obtained from Sigma (Tokyo, Japan). Para-nonylphenol, obtained from Kanto Chemicals Co., Ltd. (Tokyo, Japan) was diluted with dimethyl sulfoxide or RPMI 1640 media to a final concentration.

Jurkat cells were 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.

Forty-five µl out of 1 ml serum free conditioned medium was used for alpha-casein substrate zymography as described[15].

At 24 h incubation after adding para-nonylphenol, or para-nonylphenol with doxycycline (50 µM) in serum free RPMI 1640 media, cells were harvested, and centrifuged at 1,500 rpm for 10 min. Cell pellets and the supernatant were used for western blotting, and zymography, respectively.

After SDS gel electrophoresis, western blotting was performed using the specific anti-MMP-8 antibody and ECL chemiluminescence system (Amersham-Pharmacia, Tokyo, Japan).

Results and Discussion

The author investigated effects of para-nonylphenol on alpha-casein degrading MMP secretion by human leukemia Jurkat cells using casein-substrated zymography. Jurkat cells secrete small amount of alpha-casein degrading MMPs. As shown in Figure 1 (left panel), at 24 h incubation time para-nonylphenol (0, 0.1, 1, 5, 10, and 50 µM) dose-dependently induced the secretion of alpha-casein degrading MMPs (MW; between 40-80 kDa). As doxycycline is considered to inhibit MMP-8 activities specifically, we next studied whether the activities were inhibited by doxycycline. Jurkat cells were pretreated with 50 µM doxycycline. The casein zymography was performed. As shown in Figure 1 (right panel), the activities of casein-degrading MMPs were completely inhibited, indicating that the casein-degrading MMPs may be MMP-8.

To confirm that the casein-degrading MMP was MMP-8 we performed western blotting using the specific anti-MMP-8 antibody. As shown in Figure 2, the signals of the bands (MW; between 40-80 kDa) were detected using the ECL chemiluminescence system. The results suggest that MMP induced by para-nonylphenol was MMP-8.

In the present study the author has shown for the first time that para-nonylphenol induced the secretion of MMP-8 by human leukemia Jurkat cells. MMP-8, human neutrophil collagenase has been considered as a polymorphonuclear neutrophil-specific MMP. MMP-8 is stored in granules in cells and released from cells upon activation[13]. The role of MMP-8 was shown in chronic bronchiectasis[16], cystic fibrosis[17], and rheumatoid arthritis[18]. The expression of MMP-8 was reported to be regulated by TNF-alpha and interleukin-1[19,20]. MMP-8 is also expressed in various cell types such as fibroblasts and endothelial cells other than polymorphonuclear neutrophil.

The molecular size of MMP-8 varies between 40 kDa and 80 kDa. The variance may reflect a different degree of glycosylation. In the present study we also observed several bands between 40-80 kDa molecular size. Although we have not confirmed whether these several bands may also reflect a different degree of glycosylation of MMP-8, it is considered that several bands reflected a different degree of glycosylation of MMP-8.

Doxycycline is an antibiotics and inhibits MMP-8 activity, but not MMP-1 activity[21,22]. The mechanism of inhibition is not well understood. It was shown that long term doxycycline treatment reduced MMP-8 serum levels in reactive arthritis[23]. In the present study the casein-degrading activities were completely inhibited with doxycycline treatment, indicating that the casein-degrading MMP induced by para-nonylphenol may be MMP-8.

As MMP-8 is involved in turnover of extracellular matrix proteins, especially collagens, induction of MMP-8 secretion induced by para-nonylphenol may affect normal turnover of collagnes. Further, as MMP-8 is also involved in pathological conditions such as rheumatoid arthritis and cystic fibrosis, induction of MMP-8 secretion induced by para-nonylphenol may affect pathological conditions.

Conclusions

In conclusion, the author has shown for the first time that para-nonylphenol induced MMP-8 activity.

Acknowledgment

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

References

1. Safe, S.H. (1995) Human and Ecological Risk Assessment, 1,17.

2. Roy, D., Palangat, M., Chen, C.W., Thomas, R.D., Colerangle, J., Atkinson, A., and Yan, Z.J. (1997) J. Toxicol. Environ. Health 50, 1.
3. Colborn, T. (1995) *Environ.* Health Perspective 103, 135.
4. Danzo, B.J. (1997) Environ. Health Perspect. 105, 294.
5. Bolger, R., Wiese, T.E., Ervin, K, Nestich, S., and Checovich, W. (1998) Environ Health Perspect. 106, 551.
6. Danel, L., Souweine, G., Monier, J.C., and Saez, S. (1983) J. Steroid Biochem. 18, 559.
7. Suenaga, R., Evans, M.J., Mitamura, K., Rider, V., and Abdou, N.I (1998) J. Rheumatol. 25, 1305.
8. Massova, I., Kotra, L.P., Fridman, R., and Mobashery, S. (1998) FASEB J. 12, 1075.
9. Nelson, A.R., Fingleton, B., Rothenberg, M.L., and Matrisian, L.M. (2000) J. Clin. Oncology, 18, 1135.
10. Goldberg, G.I., Wilhelm, S.M., Kronberger, A., Bauer, E.A., Grant, G.A., and Eisen, A.Z. (1986) J. Biol. Chem. 261, 6600.
11. Hasty, K.A., Jeffrey, J.J., Hibbs, M.S., and Welgus, H.G. (1987) J. Biol. Chem. 262, 10048.
12. Freiji, J.M., Diez-Itza, L., Balbin, M., Sanchez, L.M., Blasco, R., Tolivia, J., and Lopez-Otin, C. (1994) J. Biol. Chem. 269, 16766.
13. Mainardi, C.L., Pourmotabbed, T.F., and hasty, K.A. (1991) Am. J. Med.Sci., 171, 302.
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) Mol. Cell Endocrinol. 142, 203.
15. Kubota,S., Takeda, K., Yamada, T., Nomura, Y., Takeda,M., Iwamoto, S., & Seyama,Y. (1996) Cancer Lett., 98, 233.
16. Sepper, R., Konttinen, Y. T., Ding, Y., Takagi, M., and Sorsa, T. (1995) Chest 107, 1641.
17. Power, C., O'Connor, C. M., MacFarlane, D., O'Mahonney, S., Gaffney, K., Hayes, J., and FitzGerald, M. X. (1994) Am. J. Respir. Crit Care Med. 150, 818.
18. Matsuki, H., Fujimoto, N., Iwata, K., Knauper, V., Okada, Y., and Hayakawa, T. (1996) Clin. Chim. Acta 244, 129.
19. Unemori, E. N., Hibbs, M. S., and Amento, E. P. (1991) J. Clin. Invest. 88, 1656.
20. Hanemaaijer, R., Koolwijk, P., le Clercq, L., de Vree, W. J. A., and van Hinsbergh, V. W. M. (1993) Biochem. J. 296, 803.
21. Hanemaaijer, R., Sorsa, T., Konttinen, Y., Ding, Y., Sutinen, M., Visser, H., van Hinsbergh, V.W.M., Helaakoski, T., Kainulainen, T., Ronk, H., Tschesche, H., and Salo, T. (1997) J. Biol. Chem. 272, 31504.
22. Sorsa, T., Ding, Y., Salo, T., Lauhio, A., Teronen, O., Ingman, T., Ohtani, H., Andoh, N., Takeha, S., and Konttinen, Y. T. (1994) Ann. N. Y. Acad. Sci. 732, 112.
23. Lauhio, A., Salo, T., Ding, Y., Konttinen, Y. T., Nordstrom, D., Tschesche, H., Lahdevirta, J., Golub, L. M., and Sorsa, T. (1994) Clin. Exp. Immunol. 98, 21.

Figure 1 Effects of para-nonylphenol on casein-degrading proteinase secretion by Jurkat cells

Effects of para-nonylphenol (0, 0.1, 1, 5, 10, and 50 µM) on casein-degrading proteinase secretion by Jurkat cells were analyzed using casein zymography. Serum free conditioned media were collected 24 h after the addition of para-nonylphenol. A typical zymography is shown in the left panel. The effect of doxycycline was studied. Doxycycline (50 µM) was added before the addition of para-nonylphenol (50 µM), and serum free conditioned media were collected 24 h after the addition of para-nonylphenol. The result is shown in the right panel.

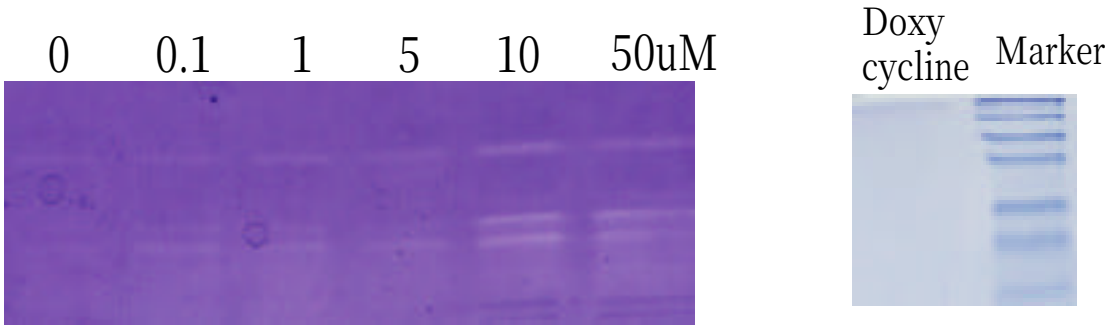

Figure 2 Western blot analysis using the specific anti-MMP-8 antibody

Western blotting using the specific anti-MMP-8 antibody was performed. The signals of the bands (MW; between 40-80 kDa) were detected using the ECL chemiluminescence system.