BIPHASIC EFFECTS OF NONYLPHENOL ON BREAST CANCER CELL FUNCTION IN VITRO

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Introduction

Considerable attention has been focused on environmental chemicals which disrupt various tissues via steroid receptor. Alkylphenols were introduced in the 1940s and have been used in pesticides, paints, herbicides and as surfactants and plastic additives. Nonylphenol is a degradation product of alkylphenol polyethoxylate detergents which exhibits estrogenic activities[1, 2]. Nonylphenol was shown to stimulate vitellogenin expression in trout hepatocytes and cell proliferation of breast cancer cell line (MCF-7) [3-6]. Environmental chemicals with estrogenic activity are considered to cause reproductive disorders, endocrine disorders, variety of cancers such as prostate, ovarian and breast cancer[7].

Mitogen activated protein (MAP) kinase is a key enzyme of the signal transduction pathways triggered by extracellular signals[8-10]. Erk1 and Erk2, which are extracellular-signal regulated kinases are ubiquitously expressed. Erk activation requires dual phosphorylation on specific threonine and tyrosine residues[8, 9]. Erk activation is mediated by a dual specific kinase termed MAP kinase/Erk kinase (MEK), which is activated by Raf oncoproteins.

Although information is available concerning growth promoting effect of nonylphenol[3-6], little is known about the effect of nonylphenol on cell death and its mechanism[11, 12]. For example, after chronic exposure to nonylphenol in adult medaka (*Oryzias latipes*), apoptosis was induced in spermatocytes, Sertoli cells and Leydig-homologue cells[11]. Estrogenic alkylphenols including nonylphenol induce cell death of testicular Sertoli cells by inhibiting testis endoplasmic reticulum calcium pumps[12]. However, it is not known whether nonylphenol induces breast cancer (MCF-7) cell death and whether estrogen receptor is involved in nonylphenol-induced cell death. It is also not known whether nonylphenol modulates MAP kinase expression.

We undertook this study to elucidate whether nonylphenol induces cell death of breast cancer cell lines, estrogen receptor-positive MCF-7 and -negative MDA-231, and whether it modulates MAP kinase expression.

Materials and Methods

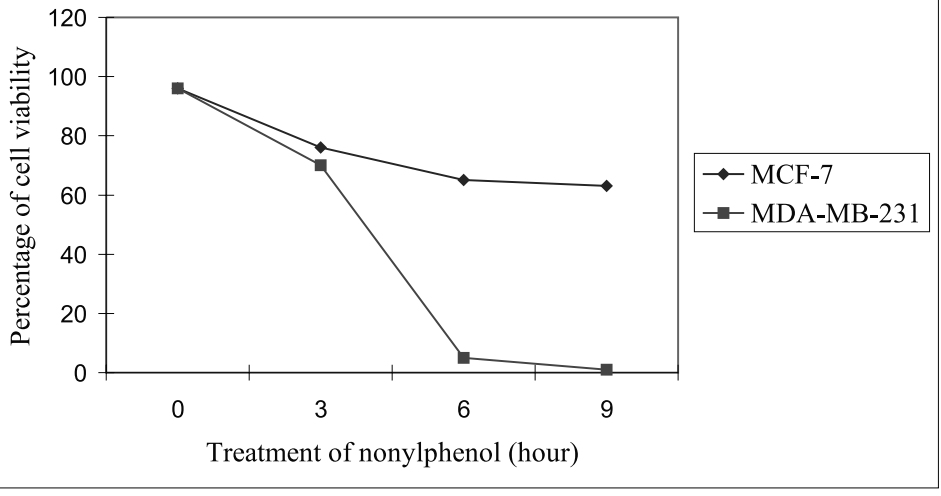
Reagents

Nonylphenol 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). Nonylphenol (0.01-100 μM) was added to subconfluent cells in 6 ml serum-free DMEM media (100 mm dishes) and incubated for 0 to 12 hours. Cells were harvested for DNA preparation and western blotting. The cell extracts were

Fig. 1 Effects of nonylphenol on breast cancer cell viability



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. The supernatant was used. Protein concentration of the supernatant was assayed using a Bradford method. The antibodies against phospho-Erk, and cytochrome c were obtained from New England Biolabs (Beverly, MA). Bradford solution for measurement of protein concentration was purchased from BioRad (Hercules, CA).

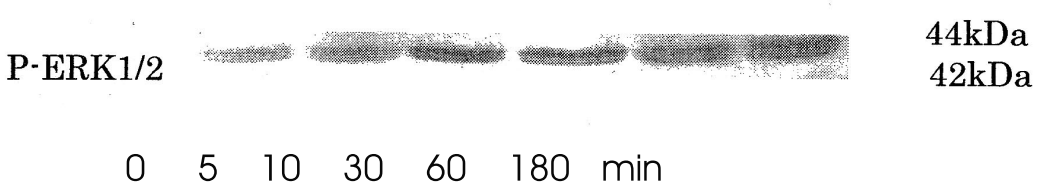
MAP kinase expression

Fifty to 100 µg of cell extracts was used. Western blotting was performed using ECL kit (Amersham Pharmacia Biotech, Buckinghamshire, UK), and anti-phosph ERK and cytochrome c antibodies.

Results and Discussion

Nonylphenol at 1-10 µM is known to stimulate cell proliferation of breast cancer cell line (MCF-7) [3-6]**.** However, it is not known whether nonylphenol induces breast cancer cell death. Therefore, we investigated the effects of nonylphenol on cell death *in vitro* using human breast cancer cell lines, estrogen receptor-positive MCF-7 and –negative MDA-231 cells. Preliminary experiment using trypan blue exclusion method showed that lower doses of nonylphenol (0.01-10 µM) didn't significantly affect cell viability of both cell lines, but higher dose (50 and100 µM) of nonylphenol induced cell death in a time-dependent manner (1-12 h). Therefore, we used 100 µM nonylphenol in the present experiment. Figure 1 shows the percentage of cell viability compared to control. Nonylphenol induced cell death of both MCF-7 and MDA-MB-231 cells. The data suggest that nonylphenol induced breast cancer cell death independent of estrogen receptor. To examine whether nonylphenol induced cell apoptosis, we first analyzed the effect of nonylphenol (100 µM) on DNA ladder formation. Nonylphenol induced DNA ladder formation in both cells (not shown). The data suggest that nonylphenol induced apoptosis of both cells independent of estrogen receptor.

Figure 2 Effect of nonylphenol on phospho ERK expression in MCF-7 cell



We next studied the mechanism of apoptosis of MCF-7 and MDA-MB-231 cells. The efflux of cytochrome c from mitochondria to cytosol was investigated by western blotting using anti-cytochrome c antibody. Cytochrome c was detected in cytosol of both MCF-7 and MDA-MB-231 cells after 3 h (not shown). The data suggest that cytochrome c efflux from mitochondria to cytosol triggers apoptosis of both cells.

It is known that ERK expression correlates with cell proliferation[8-10]. Therefore, we next studied the effect of nonylphenol (1 μM) on MAP kinase (ERK) expression (0-180 min) in MCF-7 and MDA-MB-231 cells. Nonylphenol (1 μM) induced ERK expression after 10 minutes in MCF-7 cells (Figure 2), but not in MDA-MB-231 cells (not shown). The data suggest that at lower concentration (1 μM) nonylphenol stimulated MCF-7 cell proliferation via estrogen receptor and MAP kinase (ERK) signaling pathway, but cell death is independent of estrogen receptor, and cytochrome c efflux triggers apoptosis of both cells. We are currently studying to elucidate the signal transduction of apoptosis after cytochrome c efflux in breast cancer cells.

Acknowlegement

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