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Methoxychlor and triclosan stimulates ovarian cancer growth by regulating cell cycle- and apoptosis-related genes via an estrogen receptor-dependent pathway

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ABSTRACT

Methoxychlor and triclosan are emergent or suspected endocrine-disrupting chemicals (EDCs). Methoxychlor [MXC; 1,1,1-trichlor-2,2-bis (4-methoxyphenyl) ethane] is an organochlorine pesticide that has been primarily used since dichlorodiphenyltrichloroethane (DDT) was banned. In addition, triclosan (TCS) is used as a common component of soaps, deodorants, toothpastes, and other hygiene products at concentrations up to 0.3%. In the present study, the potential impact of MXC and TCS on ovarian cancer cell growth and underlying mechanism(s) was examined following their treatments in BG-1 ovarian cancer cells. As results, MXC and TCS induced BG-1 cell growth via regulating cyclin D1, p21 and Bax genes related with cell cycle and apoptosis. A methylthiazolyldiphenyltetrazolium bromide (MTT) assay confirmed that the proliferation of BG-1 ovarian cancer cells was stimulated by MXC (10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} M) or TCS (10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} M). Treatment of BG-1 cells with MXC or TCS resulted in the upregulation of cyclin D1 and downregulation of p21 and Bax transcriptions. In addition, the protein level of cyclin D1 was increased by MXC or TCS while p21 and Bax protein levels appeared to be reduced in these cells. Furthermore, MXC- or TCS-induced alterations of these genes were reversed in the presence of ICI 182,780 (10^{-7} M), suggesting that the changes in these gene expressions may be regulated by an ER-dependent signaling pathway. In conclusion, the results of our investigation indicate that two potential EDCs, MXC and TCS, may stimulate ovarian cancer growth by regulating cell cycle- and apoptosis-related genes via an ER-dependent pathway.

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1. Introduction

Many environmental substances include natural and synthetic materials that destroy the ecosystem (Wan et al., 2011). In particular, chemicals that are harmful to the endocrine system are called endocrine-disrupting chemicals (EDCs) (Kim et al., 2012; Nurulnadia et al., 2013). EDCs have garnered extensive interest due to their industrial and domestic applications, and potential risk to human health (Cherednichenko et al., 2012; Kim et al., 2012). It has been noted that EDCs change the sexual behaviors, fertility, feminization, and reproductive organs of animals (Beronius et al., 2013; Gioiosa et al., 2013; Weber et al., 2013). More current studies demonstrated that EDCs impact human health as well (Esteban et al., 2013; Wens et al., 2013). EDCs affect the endocrine system of mammals through a variety of mechanisms including binding to steroid hormone receptors and acting on cell signaling pathways (Hwang et al., 2013; Kim et al., 2012).

Triclosan [TCS; 5-chloro-2-(2,4-dichloro-phenoxy)-phenol] is a synthetic chlorinated phenylether bisphenol used as a broad spectrum antimicrobial agent (Cherednichenko et al., 2012; Ma et al., 2013; Perez et al., 2013; Twanabasu et al., 2013; von der Ohe et al., 2012). This chemical is a common component of soaps, deodorants, toothpastes, and other hygiene products used at concentrations up to 0.3% (Cherednichenko et al., 2012; Fritsch et al., 2013; Ma et al., 2013; Middleton and Salierno, 2013; von der Ohe et al., 2012). TCS has not been classified as an EDC in the endocrine disrupter priority list, but it is considered to be an emergent or suspected EDC (Esteban et al., 2013; von der Ohe et al., 2012). Methoxychlor [MXC; 1,1,1-trichlor-2,2-bis (4-methoxyphenyl) ethane] is an organochlorine pesticide that has been primarily used since dichlorodiphenyltrichloroethane (DDT) was banned (Lee et al., 2012; Mahoney and Padmanabhan, 2010). Although MXC is less persistant than DDT in the environment, its residue in soils and sediments is still of concern (Satsuma et al., 2013). MXC has a weak estrogenicity and its active metabolite, hydroxyphenyltrichloroethane, a much more potent estrogenicity (Ye et al., 2014). MXC was revealed to exert typical endocrine-disrupting effects such as ovulation failures, uterine hypertrophy, atrophy of male sexual organs, and deteriorations of sperm production in rats and/or mice (Aly and Azhar, 2013; Aoyama and Chapin, 2014). Therefore, it may cause serious reproductive damages in other animals and humans. Ovarian cancer is curable during the early stage, but most patients are diagnosed at the end stage because of subclinical changes. Dysregulation of apoptosis and cell cycle-related genes can play an important role in the progression and pathogenesis of this disease (Atmaca et al., 2013). 17β-Estradiol (E2), a primary female sex hormone, serves as a ligand for the estrogen signaling pathway, and significantly affects the proliferation of estrogen receptor (ER)-positive cancer via ERα and ERβ (Garay et al., 2012; Louis et al., 2013; Tian et al., 2009). Specifically, ERα is known to be a primary estrogen receptor for the best-known oncogenes involved in estrogen-dependent cancers (Garay et al., 2012; Garrido et al., 2013; Lee and Choi, 2013). EDCs also influence ovarian, breast, and prostate cancers via an ERα signaling pathway and induce progression and metastasis of this type of tumors (Lee et al., 2012; Park et al., 2012; Stotter and Walker, 2010). Many studies determined that EDCs alter the transcriptional regulation of genes associated with the cell cycle (Lee et al., 2012; Park et al., 2012; Weber et al., 2013).

Members of the Bcl-2 family govern the intrinsic apoptotic pathway. This family includes both anti-apoptotic and pro-apoptotic proteins (Cao et al., 2013; Schulman et al., 2013; Su et al., 2013). Representative anti-apoptotic factors are Bcl-2, Bcl-xl, and Mcl-1, while representative pro-apoptotic proteins are Bax and Bak (Nguyen et al., 2013; Schulman et al., 2013; Su et al., 2013). Expression of Bcl-2 family protein is stimulated by an ERα signaling pathway. The expression of cell cycle-related proteins is also stimulated by an ERα signaling pathway, and many studies have suggested EDCs and E2 affect cell cycle-related proteins via the ERα (Lee and Choi, 2013; Lee et al., 2012).

In this study, we hypothesized that TCS and MXC may induce ovarian cancer cell growth by distinctly disrupting cyclin D1, p21 and Bax expressions in ER-positive BG-1 ovarian cancer cells. Thus, we examined the transcriptional and translational expressions of cyclin D1, p21, and Bax in BG-1 ovarian cancer cells following exposure to MXC and TCS in vitro. To do this, we further performed the reverse transcription (RT)-PCR and western blot analysis following their exposure in this cell type.

2. Materials and methods

2.1. Chemical preparation

All reagents including E2, MXC, TCS, and ICI 182,780 were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) and dissolved in 100% dimethyl sulfoxide (DMSO; Junsei Chemical Co., Tokyo, Japan). These chemicals were divided into 20 μl in amber microtubes and stored at room temperature during the experiments.

2.2. Cell culture and media

Human BG-1 ovarian cancer cells were obtained from Dr. K. S. Korach (National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone Laboratories, Inc., Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories), 100 U.I./mL penicillin and 100 mg/mL streptomycin (Life Technologies, Rockville, MD, USA), and 1% HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Life Technologies) at 37 °C in a humidified atmosphere of 5% CO_2 containing air. The cells were subsequently cultured in phenol-red free DMEM (DMEM; Sigma–Aldrich Corp.) supplemented with 5% charcoal-dextran-treated fetal bovine serum (FBS; Hyclone Laboratories) for 2 days before the cells were maintained with 1% charcoal/dextran-treated FBS for another 2 days to evaluate the estrogenicity of EDCs in BG-1 cells.