Triclosan (TCS) and Bisphenol A (BPA) are endocrine-disrupting chemicals (EDCs) and have a potential to increase progression of hormone responsive cancers as does 17β-estradiol (E2). 3,3′-dindolylmethane (DIM), a phytoestrogen, has been known to have anticancer activity. In this study, we examined the anti-proliferative and anti-epithelial-mesenchymal transition (EMT) effects of DIM on TCS, BPA or E2-induced EMT of MCF-7 breast cancer cells, which express estrogen receptors (ERs). In MTT assay, TCS and BPA (10^-5 M, both) induced growth of MCF-7 cells compared to a control (DMEM) like E2 (a positive control, 10^-8 M), which was antagonized by addition of ICI 182,720 (10^-8 M), suggesting that the cell proliferation effect induced by TCS appears to be mediated by an ER-dependent manner. On the contrary, DIM (20-50 μM) significantly reduced the increased viability of MCF-7 cells induced by TCS, BPA or E2. In a scratch assay, TCS and BPA also enhanced migration of MCF-7 cells like E2, but co-treatment of DM or ICI 182,720 reduced the migration ability of TCS, BPA and E2 to a control level. Along with the effect of TCS, we measured TCS, BPA or E2-induced alterations in protein expression levels of EMT related markers, i.e., E-cadherin, N-cadherin, Snail and slug by western blot assay. We detected that protein expression of E-cadherin was declined while N-cadherin, snail and slug expressions were increased by TCS, BPA or E2, indicating that TCS and BPA induced EMT process in MCF-7 cells like E2. Differently, DIM was shown to adversely affect the expression of EMT markers induced by TCS, BPA or E2. Taken together, these results present that DIM effectively inhibits the cell growth and EMT process of MCF-7 cells induced by E2, TCS or DIM. Furthermore, DIM induced metastasis genes such as catabase B and D, or they are sub genes of down stream in CXCR4 signaling pathway which stimulates EMT in ER positive breast cancer cell. Based on these in vitro results, in vivo effect of TCS, BPA and DIM on tumor growth and EMT process will be examined in a xenografted mouse model transplanted with human MCF-7 breast cancer cells. MCF-7 cells will be injected to mice and they will be taken E2, TCS, BPA and DIM chemicals in 10 weeks. And the tumor will be calculated for effects of EDCs and phytoestrogen.

ABSTRACT

Triclosan (TCS) and Bisphenol A (BPA) are endocrine-disrupting chemicals (EDCs) and have a potential to increase progression of hormone responsive cancers as does 17β-estradiol (E2). 3,3′-dindolylmethane (DIM), a phytoestrogen, has been known to have anticancer activity. In this study, we examined the anti-proliferative and anti-epithelial-mesenchymal transition (EMT) effects of DIM on TCS, BPA or E2-induced EMT of MCF-7 breast cancer cells, which express estrogen receptors (ERs). In MTT assay, TCS and BPA (10^-5 M, both) induced growth of MCF-7 cells compared to a control (DMEM) like E2 (a positive control, 10^-8 M), which was antagonized by addition of ICI 182,720 (10^-8 M), suggesting that the cell proliferation effect induced by TCS appears to be mediated by an ER-dependent manner. On the contrary, DIM (20-50 μM) significantly reduced the increased viability of MCF-7 cells induced by TCS, BPA or E2. In a scratch assay, TCS and BPA also enhanced migration of MCF-7 cells like E2, but co-treatment of DM or ICI 182,720 reduced the migration ability of TCS, BPA and E2 to a control level. Along with the effect of TCS, we measured TCS, BPA or E2-induced alterations in protein expression levels of EMT related markers, i.e., E-cadherin, N-cadherin, Snail and slug by western blot assay. We detected that protein expression of E-cadherin was declined while N-cadherin, snail and slug expressions were increased by TCS, BPA or E2, indicating that TCS and BPA induced EMT process in MCF-7 cells like E2. Differently, DIM was shown to adversely affect the expression of EMT markers induced by TCS, BPA or E2. Taken together, these results present that DIM effectively inhibits the cell growth and EMT process of MCF-7 cells induced by E2, TCS or DIM. Furthermore, DIM induced metastasis genes such as catabase B and D, or they are sub genes of down stream in CXCR4 signaling pathway which stimulates EMT in ER positive breast cancer cell. Based on these in vitro results, in vivo effect of TCS, BPA and DIM on tumor growth and EMT process will be examined in a xenografted mouse model transplanted with human MCF-7 breast cancer cells. MCF-7 cells will be injected to mice and they will be taken E2, TCS, BPA and DIM chemicals in 10 weeks. And the tumor will be calculated for effects of EDCs and phytoestrogen.

RESULTS

Figure 1. Effects of E2, TCS, BPA or DIM on cell proliferation of MCF-7 cells. MCF-7 cells were seeded at 4,000 cells/well in 96-well plates, and the presence of phenol red-free DMEM (+5% CD-FBS) containing TCS (A), DIM+TCS (B), BPA (C) and DIM+BPA (D) at the concentrations indicated for 6 days. The cell viability was determined by MTT assay. * P<0.05 compared to a vehicle treated with DMSO.

Figure 2. Effects of E2, TCS, ICI 182,720 or DIM on cell migration of MCF-7 cell migration in a scratch assay. MCF-7 cells were seeded at 6.4 × 10^4 cells/well in 24-well plates and incubated for 2 days with phenol red-free DMEM (+5% CD-FBS) before scratching with a 1ml pipette tip. Medium was replaced by phenol red-free DMEM (+5% CD-FBS) containing E2(10^-8 M), ICI 182,720(10^-8 M), DIM(10^-5 M) and DMSO (0.1%) for 0h, 24h, and 48h. E2 and TCS –induced migration was prevented by ICI 182,720 and DIM. Images were captured at X40 magnification using an Olympus CKX 41 microscope.

Figure 3. Effects of E2, TCS, ICI 182,720 or DIM on cell invasion in a transwell invasion assay. MCF-7 cells were seeded at 5 × 10^4 cells/well in 24-well plates and incubated for 2 days with phenol red-free DMEM (+5% CD-FBS) before treating with E2, TCS and DIM chemicals. Medium was replaced by phenol red-free DMEM (+5% CD-FBS) containing ICI 182,720(10^-8 M), E2(10^-8 M), ICI 182,720(10^-8 M) and DIM(10^-5 M). E2 and TCS –induced invasion was prevented by ICI 182,720 and DIM. And then it was stained with 0.5% crystal violet. Images were captured at X40 magnification using an Olympus CKX 41 microscope.

Figure 4. The expression of protein level on EMT markers for the effect of E2, TCS, ICI182,720, DIM in western blot. The expression of E-cadherin as an epithelial marker was repressed by E2 and TCS treatment compared to the control. In contrast, the expression of N-cadherin, Snail and Slug a mesenchymal marker, was significantly increased by E2 and TCS. ICI182,720 and DIM up regulated the expression of E-cadherin and down-regulated in N-cadherin and Slug in western blot. Quantiﬁcation of proteins of E-cadherin, N-cadherin, Snail and Slug were performed by scanning the densities of bands on a transfer membrane using Gel Doc 2000.

Figure 5. The expression of protein level on metastasis related markers in CXCR4 signaling pathway for the effect of E2, TCS, ICI182,720, DIM in western blot. The expression of Catepsin B and D as metastasis markers in CXCR4 signaling pathway was increased by E2 and TCS treatment compared to the control. In contrast, the expression of Catepsin B and D were significantly decreased by ICI182,720 and DIM in western blot.

Figure 6. Schedule of the experiment for xenografted mouse model in vivo. MCF-7 breast cancer cells 6 × 10^5 cells were injected to BALB/c nude mice. When tumor size reach 60~80mm³ they will be taken with E2, TCS, BPA and DIM chemicals in 11 weeks after ovarcetomy. And then tumor will be observed as effects of EDCs and phytoestrogen injection.

CONCLUSION

1. E2, TCS and BPA increased proliferation and migration ability of MCF-7 breast cancer cells.
2. Phytoestrogens, DIM, inhibited cell proliferation and migration ability of MCF-7 cells increased by E2, TCS and BPA in scratch assay.
3. In transwell invasion assay, E2, and TCS induced the invasion ability but, DIM prevented the invasion effect.
4. The expression of epithelial and mesenchymal markers were detected by E2, TCS and DIM in western blot.
5. CXCR4 signaling stimulates the EMT in breast cancer cell.
6. EDCs increased the metastasis markers such as catabase B and D via CXCR4 signaling pathway.
7. Based on these in vitro results, in vivo effect of DIM on tumor growth and EMT process will be examined in a xenografted mouse model transplanted with human MCF-7 breast cancer cells.