Triclosan-induced breast cancer growth was antagonized by kaempferol, a phytoestrogen, via regulating cell cycle, migration and apoptosis related genes in MCF-7 breast cancer cells

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ABSTRACT

Triclosan (TCS) is one of endocrine disrupting chemicals (EDCs) derived from toothpastes, deodorant and cleaning supplies. As a phytoestrogen, kaempferol (Kaem) is found in variety of vegetables. In this study, we examined anti-proliferative effects of Kaem in TCS-induced cell growth in MCF-7 breast cancer cells. In MTT assays, TCS (10^4 M) increased the cell viability of MCF-7 cells, while Kaem (50 μM) significantly reduced the cell viability compared to a control (0.1% DMSO). Kaem reversed TCS-induced MCF-7 cell growth at 50 μM. To confirm that Kaem inhibited TCS-induced cell growth, we examined the transcriptional levels of cell growth and apoptosis-related markers using reverse transcription (RT)-PCR. The expression levels of cyclin D, cyclin E and Bax were increased, while that of p21 and bax mRNAs was decreased in TCS in MCF-7 cells. In addition, Kaem treatment significantly reversed TCS-induced gene expressions. In parallel with its mRNA levels, the protein level of cyclin E, cyclin D, c-Jun, c-Jun, p-IRS1, p-Akt, p-Mek1/2 and p-ERK proteins were induced by TCS while it was reversed by Kaem. The expression levels of p21 and bax genes was altered by TCS and reversed by Kaem treatment. For in vivo assay, a xenografted mouse model was generated following injection with MCF-7 breast cancer cells. In parallel with in vitro results, tumor volumes following treatment with E2 and TCS were continually increased compared to a vehicle (corn oil). It was of interest that treatment of the mice with combination of E2 plus Kaem or TCS plus Kaem showed less tumor formation rather than that of single treated mice with E2 or TCS. Taken together, these results indicate that Kaem may inhibit the growth MCF-7 cells via regulating the expression of cell cycle, migration and apoptosis-related genes, suggesting that TCS-induced progression of breast cancer may be suppressed by a phytoestrogen.

RESULTS

Figure 1. Viability of MCF-7 breast cancer cells following treatment with chemicals. Cells were treated with DMSO (vehicle), E2 (10^-9M), TCS (10^-6M), Kaem (50 μM) were treated. Total RNA and proteins were extracted after the treatment period (A) DNA bands of cyclin D,cyclin E, p21 and GAPDH genes were detected by using Reverse transcription PCR (A). Western blot analysis were used to measure protein levels of cyclin D, cyclin E, p21 and GAPDH. Data represent the means ± S.D. of triplicate experiments. *: a significant elevation or reduction comparing with control treated with DMSO (p < 0.05 in Dunnett’s multiple comparison test).

Figure 2. Altered protein expression levels of IGF related gene following treatment with chemicals. DMSO (vehicle), E2 (10^-9M), TCS (10^-6M), Kaem (50 μM) were treated. Total RNA and proteins were extracted after the treatment period (A) DNA bands of insulin receptor substrate-1 (IRS-1), Akt, Mek1/2 and ERK genes were detected by using Reverse transcription PCR (A). Western blot analysis were used to measure protein levels of IRS-1, Akt, Mek1/2 and ERK. Data represent the means ± S.D. of triplicate experiments. *: a significant elevation or reduction comparing with control treated with DMSO (p < 0.05 in Dunnett’s multiple comparison test).

Figure 3. Altered expression levels of cell cycle related genes following treatment with chemicals. DMSO (vehicle), E2 (10^-9M), TCS (10^-6M), Kaem (50 μM) were treated. Total RNA and proteins were extracted after the treatment period (A) DNA bands of cyclin D, cyclin E, p21 and GAPDH genes were detected by using Reverse transcription PCR (A). Western blot analysis were used to measure protein levels of cyclin D, cyclin E, p21 and GAPDH. Data represent the means ± S.D. of triplicate experiments.

Figure 4. Altered expression levels of apoptosis related genes following treatment with chemicals. DMSO (vehicle), E2 (10^-9M), TCS (10^-6M), Kaem (50 μM) were treated. Total RNA and proteins were extracted after the treatment period (A) DNA bands of Bax and GAPDH genes were detected by using Reverse transcription PCR (A). Western blot analysis were used to measure protein levels of Bax and GAPDH. Data represent the means ± S.D. of triplicate experiments.

Figure 5. Effect of E2, TCS, and Kaem on cancer proliferation in animal models. (A) The schedule of the animal experiments. MCF-7 cells were transplanted to 6 week old female nude mice to manufacture breast cancer xenograft models. After the tumor formation (55 mm³ in tumor volume) the mice were treated in the presence of absence of Kaem (100 mg/kg b.w.) for 6 weeks.*: a significant elevation or reduction comparing with control treated with DMSO (p < 0.05 in Student’s t-test).

Figure 6. Effect of E2, TCS, and Kaem on cancer proliferation in animal models. (A) Tumor volume for 6 weeks. (B) Histological observation of tumor tissues by hematoxylin and eosin staining. (C) Immunohistological images of PCNA in each protein was observed under a light microscopy (magnification ×100).

Figure 7. The anti-proliferative activity of Kaem in estrogen-dependent MCF-7 breast cancer treated with E2 or TCS. Kaem has a definite antiproliferative activity by antagonizing the mitochondrial DNA and IGF-1R signaling pathways and MCF-7 breast cancer proliferation. On the contrary, Kaem is a phytoestrogen effective in modifying breast cancer progression by modulating cell cycle, apoptosis and metastasis related genes that induced by E2 or TCS.

Conclusion

In conclusion, our findings provide a more specific mechanism that TCS and E2 induce MCF-7 breast cancer proliferation through ER or IGF signaling pathways. And promote cancer progression by modulating the activities of cell cycle, apoptosis and metastasis related genes. In addition, we demonstrated that as a novel phytoestrogen, Kaem effectively act as a chemopreventive agent by suppressing carcinogenesis risks induced by TCS and E2.

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