Treatment with antifungal agents, fenhexamid and cyprodinil, resulted in an increase of cell cycle- and metastasis-related genes in an estrogen receptor-dependent pathway in cellular and xenografted mouse models with BG-1 ovarian cancer cells

Ryeo-Eun Go and Kyung-Chul Choi

Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea

Abstract

Fenhexamid and cyprodinil are antifungal agents used in agricultural applications and present at measurable amounts in fruits and vegetables. In this study, the effects of fenhexamid and cyprodinil on ovarian cancer cell viability and metastasis were examined and the expression levels of proteins such as cyclin D1 and E, and cathepsin B and D were analyzed in BG-1 ovarian cancer cells with estrogen receptors (ERs). BG-1 cells were cultured with 0.1% DMSO (control), 10-δestradiol (E2; 1 x 10^{-10} M), fenhexamid or cyprodinil (10−10 M). As results, in MIT assay, E2 as a positive control markedly increased BG-1 cell viability about 5 times and these antifungal agents increased BG-1 cell viability about 1.5 to 2 times compared to control. When the respective treatment was co-treated with ICI 182,780, an ER antagonist, BG-1 cell viability was reduced to the level of control. In wound-healing scratch assay, the scratched area was reduced by BG-1 cells treated with E2 or these antifungal agents compared with control. However, when BG-1 cells were treated with ICI 182,780, the increased protein levels were reversed. In xenografted mouse models transplanted with BG-1 cells, E2 significantly increased the tumor mass formation about 6 times and cyprodinil also induced tumor formation about 2 times compared to vehicle (0.1% DMSO) during 80 days. However, fenhexamid did not increase the tumor mass formation. These results imply that the fenhexamid and cyprodinil may have disruptive effects on ER expressing cancer by alteration of cell cycle- and metastasis-related genes via ER dependent pathway.

Key words: Endocrine disruption; cell cycle gene; metastasis gene; fenhexamid; cyprodinil; ovarian cancer

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Result

Figure 1. Cell growth by E2, Fenhexamid, Cyprodinil and co-treatment ICI 182,780 with E2 or Fenhexamid in BG-1 cells for 8 days, and the number of viable cells was measured using MIT assay at 540nm. Data represent the mean ± SD of quintuple experiment. *P<0.01 compared with vehicle, 0.1% DMSO.

Figure 2. Expression of CyclinD1 protein by treatment of E2, Fenhexamid, Cyprodinil and/or ICI182780 in BG-1 cells for the time-dependent. Protein was isolated and protein levels were assayed by Western Blot. Data represents the mean ± SD of quintuple experiment. *P<0.01 compared with each 0h of group.

Figure 3. Expression of CyclinD1 protein by treatment of E2, Fenhexamid, Cyprodinil and/or ICI182780 in BG-1 cells at 72h. Protein was isolated and protein levels were assayed by Western Blot. Data represents the mean ± SD of quintuple experiment. *P<0.01 compared with each 0h of group.

Figure 4. Expression of CyclinE protein by treatment of E2, Fenhexamid, Cyprodinil and/or ICI182780 in BG-1 cells for the time-dependent. Protein was isolated and protein levels were assayed by Western Blot. Data represents the mean ± SD of quintuple experiment. *P<0.01 compared with each 0h of group.

Figure 5. Expression of CyclinF protein by treatment of E2, Fenhexamid, Cyprodinil and/or ICI182780 in BG-1 cells at 72h. Protein was isolated and protein levels were assayed by Western Blot. Data represents the mean ± SD of quintuple experiment. *P<0.01 compared with vehicle, 0.1% DMSO.

Figure 6. Representative H & E staining images and immunohistochemical images of PCNA and Catenalin in the isolated tumors. The tumors were excised from each treatment group (0.1% DMSO, Fenhexamid, Cyprodinil and BG-1 ovarian cancer xenograft mice after sacrifices and then embedded in paraffin. Paraffin blocks were cut into 5 μm thick sections and each section was treated with primary antibody by IHC staining protocol for measuring the immunohistochemical images of H & E staining (A), PCNA (B) and Catenalin (C). (magnification, 200 x or 100 x).

Discussion

1. To evaluate the ability of pesticides stimulate cell proliferation, BG-1 cells were cultured with vehicle (0.1% DMSO), E2 (1 x 10^{-9} M), pesticides (1 x 10^{-9} M to 1 x 10^{-6} M), E2 as a positive control markedly increased BG-1 cell proliferation compared to 0.1% DMSO, pesticides gradually increased the proliferation of BG-1 cells in a 1 x 10^{-9} M to 1 x 10^{-6} M compared to vehicle.

2. To investigate the involvement of ERs action, BG-1 cells were treated with either pesticides or E2. Then co-treatment of ICI 182,780, IC182780, significantly reduced the cell growth stimulated pesticides or E2.

3. These results showed that the proliferation of BG-1 cells is mediated by an ER-dependent pathway involving ER-alpha.

4. To evaluate the effect of E2, pesticides or/and IC182780 on Protein expression of CyclinD1 and CyclinE by Western Blot. The protein expression of CyclinD1 and CyclinE was increased in E2 and pesticides for 72h, but reversed by co-treatment ICI 182,780.

5. To evaluate the effect of pesticides or/and IC182780 on cell viability by Wound-healing assay. Scratch assay of cells were significantly closed in E2 and pesticides for 48h, but reduced the ability to close by co-treatment ICI 182,780.

6. To evaluate the effect of E2, pesticides or/and IC182780 on protein expression of CyclinD1 and CyclinE by Western Blot. The protein expression of CyclinD1 and CyclinE was significantly increased in E2 and pesticides for 72h, but reduced by co-treatment ICI 182,780.

7. In xenografted mouse models transplanted with BG-1 cell, E2 and pesticides significantly stimulated the growth of tumor.

8. These study indicated that three pesticides increased the expression level of CyclinD1, CyclinE and Catenalin. But by ER antagonist, IC182780. In the other words, expression of CyclinD1, CyclinE and Catenalin have a ability to grow tumor growth or metastasis correlation with ER pathway in BG-1 ovarian cancer cell.

Reference