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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

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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 cancer cell viability and metastasis were examined and the expression levels of proteins such as cyclin D1 & E, and cathepsin B & D were analyzed in BG-1 ovarian cancer cells with estrogen receptors (ERs). BG-1 cells were cultured with 0.1% DMSO (control), 17β-estradiol (E2; 1 x 10^{-9} M), fenhexamid or cyprodinil (10^{-5} - 10^{-8} M). As results, in MTT 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 reversed 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 scratched area was maintained to the level of control. Protein levels of cyclin D1 & E, and Cathepsin B & D were induced by E2 and these antifungal agents, but when co-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

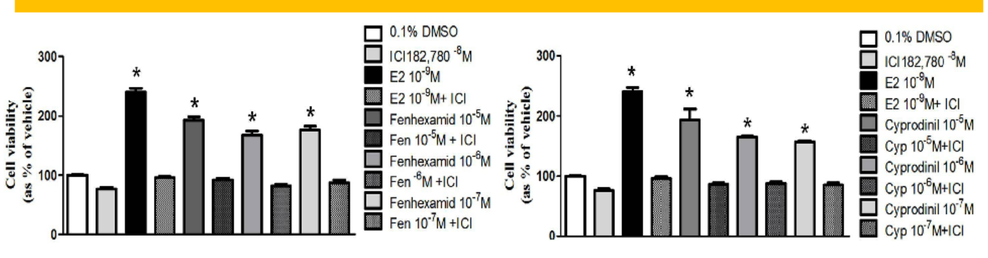

Figure 1. Cell growth by E2, Fenhexamid, cyprodinil and co-treatment ICI 182,780 with E2 or Fenhexamid in BG-1 cells for 8 days, and the number of viable cells was measured using MTT assay at 540nm. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with vehicle, 0.1% DMSO .

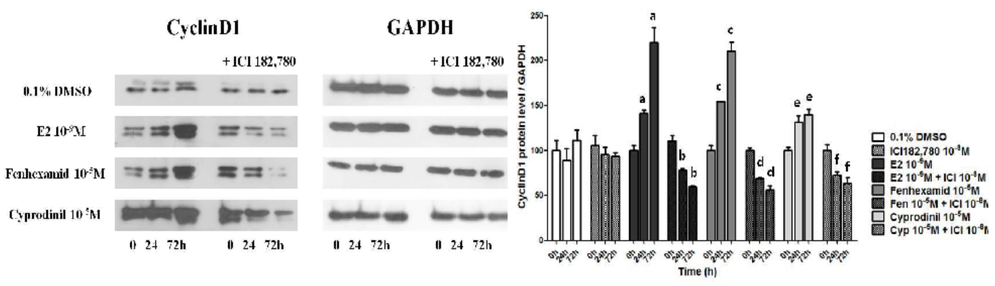

Figure 2. Expression of CyclinD1 protein by treatment of E2, Fenhexamid, Cyprodinil or/and ICI182,780 in BG-1 cells for the time-dependent. Protein was isolated and protein levels were assayed by Western Blot. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with each 0h of group.

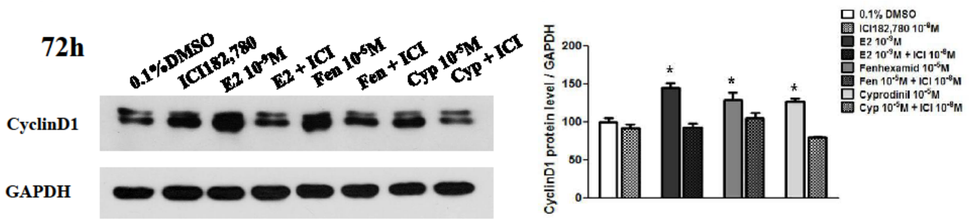

Figure 3. Expression of CyclinD1 protein by treatment of E2, Fenhexamid, Cyprodinil or/and ICI182,780 in BG-1 cells at 72h. Protein was isolated and protein levels were assayed by Western Blot. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with vehicle, 0.1% DMSO .

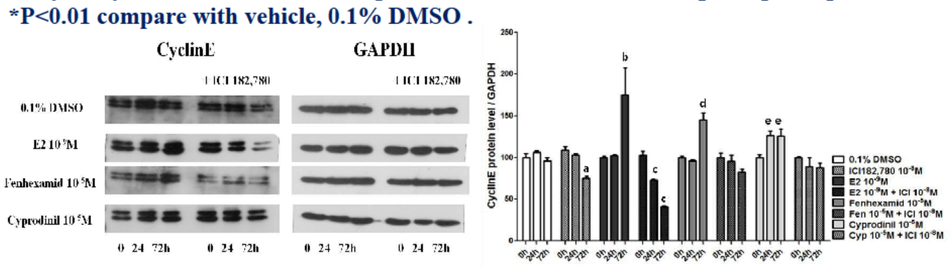

Figure 4 Expression of CyclinE protein by treatment of E2, Fenhexamid, Cyprodinil or/and ICI182,780 in BG-1 cells for the time-dependent. Protein was isolated and protein levels were assayed by Western Blot. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with each 0h of group.

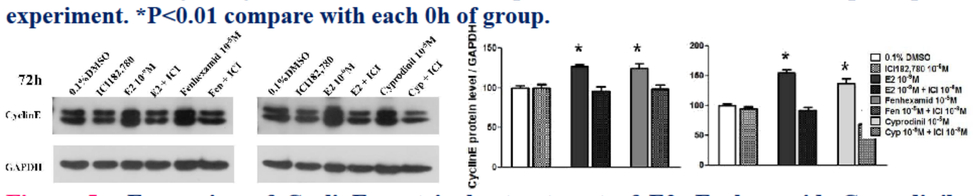

Figure 5. Expression of CyclinE protein by treatment of E2, Fenhexamid, Cyprodinil or/and ICI182,780 in BG-1 cells at 72h. Protein was isolated and protein levels were assayed by Western Blot. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with vehicle, 0.1% DMSO .

Result

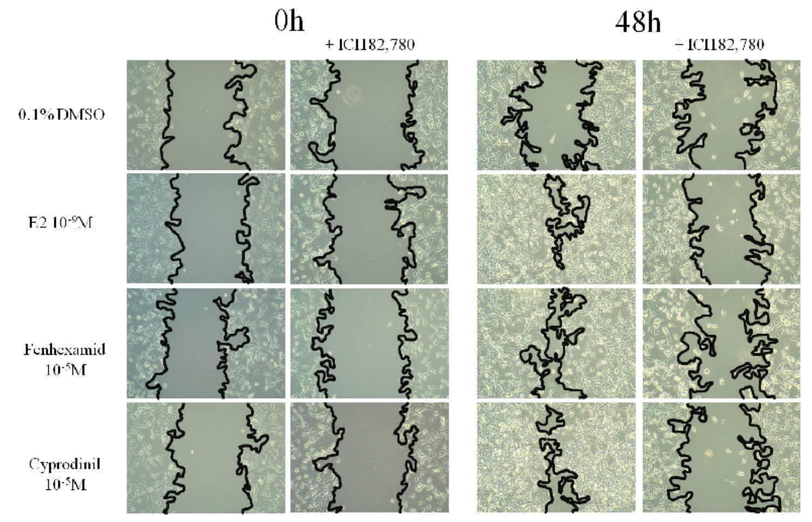

Figure 7. Cell metastasis by E2, Fenhexamid, Cyprodinil and co-treatment ICI 182,780 with E2 Fenhexamid or Cyprodinil in BG-1 cells for 48h.

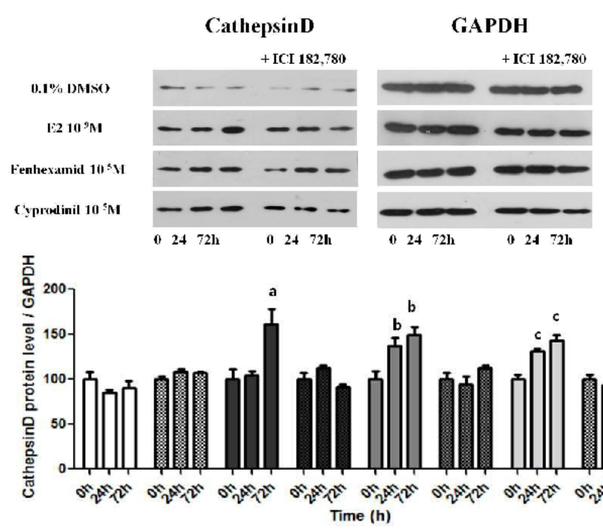

Figure 8. Expression of CathepsinD protein by treatment of E2, Fenhexamid, Cyprodinil or/and ICI182,780 in BG-1 cells for the time-dependent. Protein was isolated and protein levels were assayed by Western Blot. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with each 0h of group.

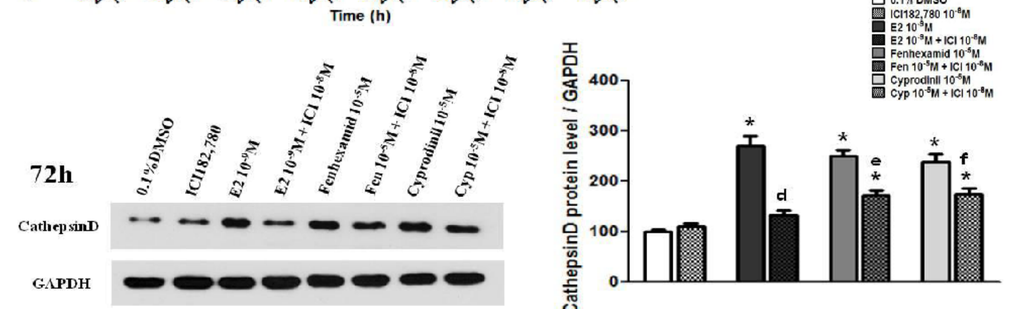

Figure 9. Expression of CathepsinD protein by treatment of E2, Fenhexamid, Cyprodinil or/and ICI182,780 in BG-1 cells at 72h. Protein was isolated and protein levels were assayed by Western Blot. Data represent the means ± SD of quintuple experiment. *P<0.01 compare with vehicle, 0.1% DMSO .

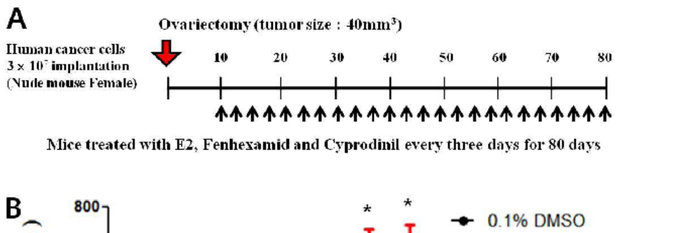

Figure 10. Effect of E2, Fenhexamid and cyprodinil on the tumor growth in the absence of endogenous estrogen. The mice were injected i.p. with DMSO, E2, fenhexamid and cyprodinil every 3 days (A) and tumor volumes were measured by length x width x height x 0.5236 (mm3) using a caliper every 10 days during the experiment period of 80 days (B). * : Mean values were significantly different from DMSO (vehicle), P<0.05. (Student's t-test).

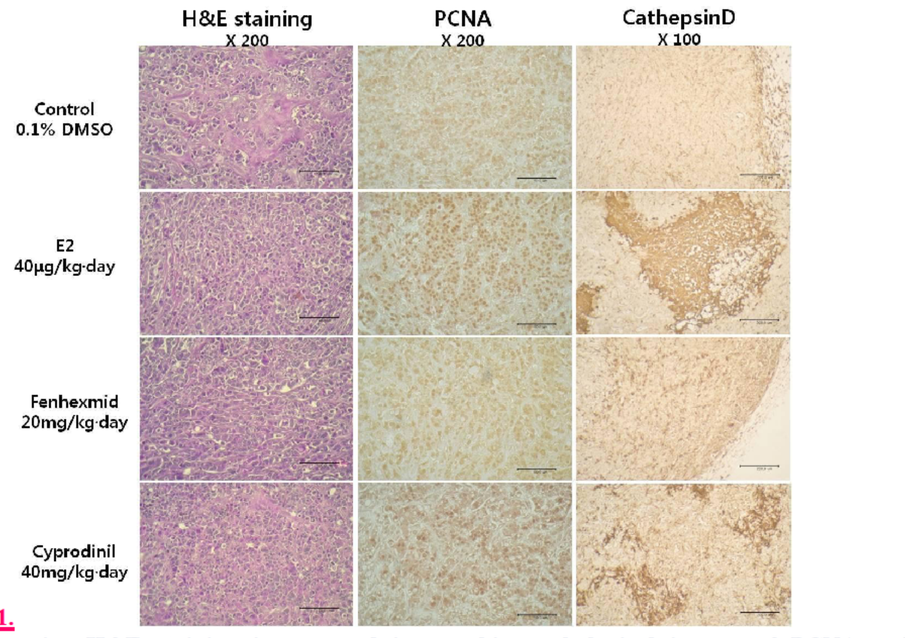

Figure 11. Representative H&E staining images and immunohistopathological images of PCNA and CathepsinD proteins in the isolated tumors. The tumor tissues were excised from each treatment group (0.1% DMSO, E2, fenhexamid and cyprodinil) of BG-1 ovarian cancer xenografted mice after sacrifice and then embedded in paraffin. Paraffin blocks were cut in into 5 μm thick sections and each section was treated with primary antibody by IHC staining protocol for measuring the immunohistological images of H&E staining (A), PCNA (B), and CathepsinD1 (C). (magnification, 200 x or 100 x).

Conclusion

1. To evaluate the ability of pesticides stimulate cell proliferation, BG-1 cells were cultured with vehicle (0.1% DMSO), E2 (1x 10^{-8}M) , pesticides (1x 10^{-5}M – 1x10^{-7}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 1x10^{-5}M to 1x10^{-7}M compared to DMSO.
2. To investigate the involvement of ERs pesticides action, BG-1 cells were treated with either pesticides or E2, and then co-treated with ICI182,780. ICI182,780 significantly repressed 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 ICI182,780 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 E2, pesticides or/and ICI182,780 on cell metastasis by Wound-healing assay. Scratch of cells were significantly closed in E2 and pesticides for 48h, but reduced the ability to restore by co-treatment ICI 182,780.
6. To evaluate the effect of E2, pesticides or/and ICI182,780 on protein expression of CathepsinD by Western Blot. The protein expression of CathepsinD were significantly increased in E2 and pesticides for 72h, but reduced by co-treatment ICI 182,780.
7. In xenograft mouse models transplanted with BG-1 cell, E2 and pesticides significantly stimulated the growth of tumor.
8. These study indicated that E2 and three-pesticides increased the expression level of CyclinD1, CyclinE and CathepsinD. But reversed by ER antagonist, ICI182,780. In the other words, expression of CyclinD1, CyclinE and CathepsinD have a ability of tumor growth or metastasis correlation with ER pathway in BG-1 ovarian cancer cell.

Reference

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