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Requirement of Metabolic activation of Pueraria mirifica for Estrogenic activity

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Introduction

The steroid hormones influence the growth, differentiation, and functioning of many target tissues. Estrogens also play an important role in bone maintenance, in the central nervous system and in the cardiovascular system where estrogens have certain cardioprotective effects (Turner et al., 1994; Farhat et al., 1996; Iafrati et al., 1997).

Human diet contains several plant-derived, nonsteroidal weakly estrogenic compounds (Korach et al., 1994). Phytoestrogens act as weak mitogens for breast tumor cells in vitro, and they may act as chemopreventive agents is suggested by the fact that intake of phytoestrogens is significantly higher in countries where the incidence of breast and prostate cancers is low (Messina et al., 1994).

Pueraria mirifica (PM) is an indigenous herb of Thailand, known in Thai as Kwao Kreu or Kwao Kreu Kao (White Kwao Kreu). Similar to soybean, it belongs to the same subfamily and possesses several compounds that act as phytoestrogens such as phenol miroestrol and deoxymiroestrol (Chansakaow et al., 2000).

In the present study, we have evaluated estrogenic activity of PM in recombinant yeast assay stably expressing human estrogen receptor (hER) and corresponding -galactosidase reporter gene, in MCF-7 human breast cancer cells proliferation assay, and in transient trasfenction assay using HepG2 human hepatoma cells in which an estrogenic response is created by cotransfection with recombinant rat ER cDNA in the presence of an estrogen-dependent luciferase reporter plasmid (C3-luc).

Materials and Methods

Materials

17-estradiol (E2) and 4-hydroxytamoxifen (OHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pueraria mirifica (PM) as test material was obtained from Cheil Jedang (In-chon, Korea). All the test materials were dissolved in the appropriate solvents for each experiment.

Methods

1. *Recombinant yeast assay*

1-1. *Recombinant yeast cell*

Saccharomyces cerevisiae ER+LYS8127 were obtained from Dr. Donald P. McDonnell(Duke University Medical Center, USA).

1-2. *Estrogenicity assay in yeast*

Yeast cells were diluted in selective medium and treated. After incubation for 18hr, yeast culture samples were diluted in the appropriate selective medium to an OD600nm. The OD420nm and OD590nm values of each well were measured using ELISA

2. MCF-7 cell proliferation assay

The cells were plated in phenol red free D-media supplemented 5% fetal bovine serum(FBS) for 24hr and replaced in 5% dextran-charcol-coated (DCC) FBS for 24 hr. Then the medium was removed and replaced by test medium. The cells were lysed, then OD260nm value of the clear lysate was measured with a spectrophotometer.

3. *Transient transfection assay in HepG2 cell*

3-1. *Plating and transfection*

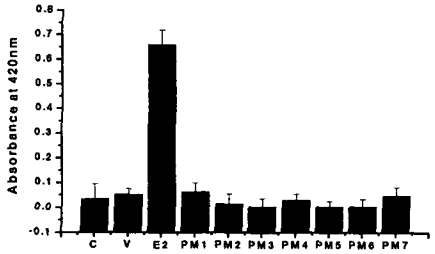
HepG2 human hepatoma cells (Korean Cell Line Bank, Korea) were plated in phenol red-free EMEM supplemented with 10% DCC-FBS for 24 hr. Transfected following the Superfect procedure (Qiagen, Valencia, CA) with two plasmids: (1) 0.4g/ml receptor plasmid encoding rat ER, (2) 0.8g/well C3-luc, reporter plasmid. Transfected cells were treated for 24 hr and then lysed.

3-2. *Dual Luciferase reporter assay.*

Lucifease assay reagent II (Promega, Madison, WI, USA) was added into each well and then firefly luciferase activity was determined immediately using microplate luminometer LB96P (Berthold technologies, Germany).

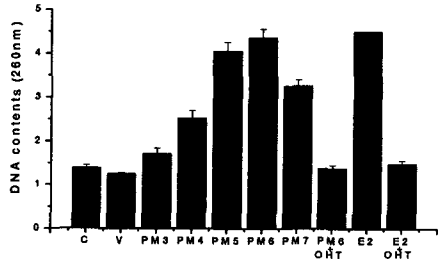
Results and Discussion

1. *Recombinant yeast assay*



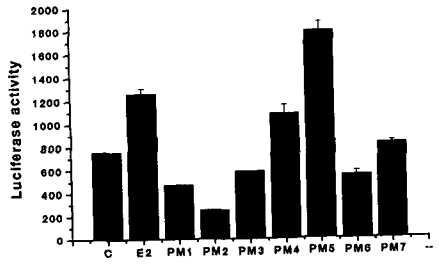
We employed a two-plasmid system consisting of human Estrogen repetor (hER) expression plasmid and a reporter plasmid containing estrogen response element (ERE) to study estrogenic property of PM (Fig. 1). PM was tested at the concentration of 0.025, 0.25, 2.5, 25, 2.5×10^2, 2×10^3, 2×10^4 ng/ml compared with 10^{-9}M of E2 as positive control. The reporter gene -galactosidase gave a measure for ligand-dependent transactivation. In recombinant yeast assay, 0.025, 0.25, 2.5, 25, 2.5×10^2, 2.5×10^3, 2.5×10^4 ng/ml concentrations of PM did not induce any estrogenic activities while 10^{-9} of E2 as positive control had strong estrogenic activity.

2. *MCF-7 cell proliferation assay*



The estrogenic activity of PM was estimated in terms of its proliferation-promoting effects in MCF-7 human breast cancer cells (Fig. 2). Estrogenic activity was observed from 2.5 ng/ml concentration of PM in a dose-dependent manner. The PM concentration of maximal estrogen activity was 2.5×10^3 ng/ml and it exhibited strong proliferation similar to E2 at the concentration of 2.5×10^{10} M. DNA contents also decreased as low as the level of vehicle control when OHT, estrogen receptor antagonist, was co-treated with PM in a dose of 2.5×10^3 ng/ml, maximal effective concentration of PM or 2.5×10^{10} M of E2, respectively.

3. *HepG2 cell transient transfection assay*



We characterized the estrogenic activity of PM in HepG3 human hepatoma cells transfected with rER plus an estrogen-responsive luciferase reporter gene (Fig. 3). The estrogenic activity of PM in HepG2 cell was similar to that of PM in MCF-7 cells. PM was complete agonists at the ER and 2.5×10^3 ng/ml of PM, maximal effective concentration, was able to show stronger estrogenic activity than E2 at the concentration of 10^{-8} M.

Acknowledgement

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Reference

Chansakaow S., Ishikawa T., Sekine K., Okada M., Higuchi Y., Kudo M., Chaichantipyuth C., 2000. Isoflavonoids from Pueraria mirifica and their estrogenic activity. Planta Med 66(6), 572-575.

Chansakaow S., Ishikawa T., Seki H., Sekine K., Okada M., and Chaichantipyuth C., 2000. Identification of deoxymiroestrol as the actual rejuvenating principle of Kwao Keur . Pueraria mirifica. The known miroestrol may be an artifact. Journal of Natural Products 63(2), 173-175.