Assessment of phytochemicals for the estrogenic activity using ERE-MCF-7

J.E. Joung, Y.W. Kim, S.R.Bang, K.N.Min and Y.Y. Sheen
College of Pharmacy, Ewha Womans University, Seoul
120-750, Korea,

Introduction

Human and ecological systems are exposed to various estrogenic chemicals from the environment, some of them are phytoestrogens originated from plants, some of them are mycoestrogens originated from microoganism, some of them are industrialestrogens, primarily alkylphenols. These environmental estrogens are not steroidal chemicals but still be able to interact estrogen receptors and to transactivate ERE in estrogen target genes(Milligan et al 1998, Martin et al 1978, Villalobos et al 1995, Kupier et al 1998). Phytoestrogens, such as coumesterol and genistein treated rat showed the uterotophic effect and 1000 fold concentrations of nonylphenol and octylpheol compared to estradiol treatment to MCF-7 cells showed cell proliferative effect comparable to that of estradiol treatment(Ratnasabapathy et al 1997, White et al 1994, Soto et al 1991). Phytoestrogens, such as genistein, daidzein, apigenin, naringenin, coumesterol were able to inhibit the estrogen binding to estrogen receptor with 100 to 1000 fold concentrations that of estradiol based on competition assay and 1-10 nM phytoestrogens were able to transactivate estrogen responsive gene activation via specific interaction with estrogen receptor (Ciolino et al 1998, Hsieh et al 1998). In this study, in order to establish the rapid and easy-to-perform methods to screen estrogenic activity of many compounds, we determined 5'ERE-regulated transactivation and cell proliferation in MCF-7 cells by luciferase assay and SRB assay, respectively.

Methods and Materials

Cell culture and transfection

MCF-7 human breast cancer cells were transfected with pERE-Luc, pSVNEO and LipofecTAMINE according to supplier's manual. And stably transformed cells were selected using G418.

Chemical treatment

MCF-7 cells were rinsed with serum-free medium twice before the administration of various chemicals in serum free medium. Stock solutions of chemicals were made in DMSO as a vehicle and control cells were treated with 0.1% DMSO

Luciferase reporter assay

The luminescents were measured using luciferin.

Cell proliferation assay

MCF-7 cells were seeded into 96 well plate and treated with various chemicals in -phenol red -serume media. SRB assay was carried out at 570 nm.

Results and Discussion

MCF-7 stable cells which are stably transfected with phERE-Luc were treated with many chemicals and then luciferase activity were determined. Estradiol (E2) and synthetic estrogen, diethylstylbesterol (DES) were induced luciferase activity in dose dependent manner and their induced activities were decreased by tamoxifen (Tam) treatment. Curcumin derivatives, such as SB118, SB123, induced the luciferase activity and Tam treatment decreased SB118-and SB123-induced luciferase activities. Other curcumin derivative, SB100, didn't induce the luciferase activity. Over than 30 flavonoids were tested in this system, and isoflavone, such as biochanin A, daidzein, genistein, showed higher luciferase activity than others. Resveratrol driven from red wine induced the luciferase activity in dose dependent manner. To determine cell proliferative effect of chemicals, SRB assay was performed. E2 and DES increased the SRB readings 20-30 folds over that of control, and their activities were blocked by Tam treatment. 29 Flavonoids and 5 curcumin derivatives were tested in this system, but only 7 compounds elicited the significant cell proliferative effect. Their E2 equivalent concentrations (EEQs) were calculated as a concentration of E2 that resulted in the same SRB reading of test compound from the dose response curve. These data show that these methods are valuable tools for screening estrogenic activity of chemical. We also have examine the estrogenic like activites of a large series of flavonoids and there seems like some relationship between their structure and activity. First, 4-methoxylation and catechol structure decreased estrogenic activities. Second, hydroxylation of 3 position reduced estrogenic effect. Third, glycosides of flavonoids showed weak estrogenic activity or no activity. Interestingly, when tested at high concentrations, genistein, kaempferol, biochanin A and chrysin elicited luciferase induction higher than that of the maximum induction by estradiol. And these effect of genistein and kaempferol could not be fully inhibited with tamoxifen.

Acknowlegement. This study was supported by 2000 KFDA EDC Grant and G7 Grant from the Ministry of Environment of Korea]

References

Ciolino, H. P. Daschner, P. J., Wang, T. T. Y. and Yeh, G. C. Effect of curcumin on the aryl

hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. *Biochem. Pharmacol.*, **56**, 197-206 (1998)

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J-A. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Porc. Natl. Acad. Sci. USA*, **93**, 5925-5930 (1996)

Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., van der Saag, P. T., van der Burg, B. and Gustafsson, J- . Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinology*, **139**, 4252-4263 (1998)

Miksicek, R. J. Estrogenic flavonoids:structural requirements for biological activity. *Proc. Soc. Exp. Biol. Med.*, **208**, 44-50 (1995)

White, R., Jobling, S. A., Hoare, J. P., Sumpter and Papker, M. G. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology.*, **135**, 175-182 (1994)