Journal of Korean Association
of Cancer Prevention
2002; 7(4): 268-273

Estrogen Like and Antiestrogenic Activity of Genistein in Cultured Cell Lines and Female Rats

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= Abstract =

Genistein is a phytoestrogen of the 'isoflavone' class compounds. These diphenolic compounds resemble the structure of estradiol and have been shown to have weak estrogenic activity. It appears that they have both estrogenic and antiestrogenic effects, depending on the concentrations of circulating endogenous estrogens and estrogen receptor (ER). In cultured cell lines, the effects of different concentrations of genistein, estrogen or in combination with genistein and estradiol on p21, p53, cyclin B_1, bax and bcl_2 expressions were examined. At the low concentrations, genistein showed estrogen agonist action with p21 and p53 expressions. But at the higher concentrations, genistein had antiestrogenic activity increasing expression of these proteins in the presence of estrogen. The increased expression of cyclin B_1 by estrogen was tampered by genistein at the highest concentration. Genistein increased the expression of bax at the three concentrations tested. In the presence of estrogen, genistein showed estrogen agonistic reaction as for bax protein. The expression of bcl_2 protein did not appear to be influenced by either genistein or estrogen. In estrogen-deficient female rats the two different concentrations of genistein were employed along with estrogen treatment. The expressions of p21, pp53 and bax were examined. *In vivo* system, estrogen agonistic activity was shown with the higher concentration of genistein with bax expression. In Cyclooxygenase-2 (COX-2) expression, the lower concentration of genistein showed estrogen agonistic activity. The inhibitory effect of genistein on the proliferation of cultured cells supports the hypothesis that this compound may play a role in the prevention of particular types of cancers.

Genistein (4',5,7-trihydroxyisoflavone), the major phytoestrogen in soybeans, legumes, has a structural similarity to the estrogens. The molecular distances between the two-OH groups on equol nucleus of genistein and those of 17 beta-estradiol are similar and OH groups offer strong binding affinity to the estrogen receptor (Brzozowski et al., 1997).

Epidemiological evidence indicates that there are

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한글제목 및 저자: Genistein의 배양세포와 암쥐에서의 에스트로겐 유사 또는 에스트로겐 길항작용에 관한 연구
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positive associations between chemoprevention and dietary soy consumption. A cross-national study involving 50 countries identified soy products as having a highly significant protective effect against prostate cancer (Herbert et al., 1998). Asian women with high soy intake have a low incidence of breast cancer (Adlercreutz et al., 1991, Lee et al., 1991) and the protection by soy intake is lost following adoption of a Western-based diet in second generations emigrating to the US (Ziegler et al., 1993). Furthermore, urinary levels of phytoestrogens were lower in breast cancer cases compared with case-controls (Ingram et al., 1997, Zheng et al., 1999). The association between dietary soy and lower incidence of cancer of the endometrium has also been suggested (Goodman et al., 1997).

Earlier studies have focused on the antiestrogenic activity of genistein for the mechanism of chemopreventive action. Interference at the level of estrogen receptor suggested to be a major role of genistein in inhibition of tumor-promoting effect of estrogens (Shutt et al., 1972, Martin et al., 1978). The initial observation (Akiyama et al., 1987) that genistein is a specific inhibitor of tyrosine protein kinase, an enzyme frequently over-expressed in cancer cells sparked many mechanistic studies of genistein on chemoprevention. Genistein is known to inhibit the growth of hormone-dependent and independent cancer cells in vitro with IC_{50} 10 to 50 micromol/L (Peterson and Barnes, 1991; Pagliacci et al., 1994; Peterson and Barnes, 1996; Hsieh et al., 1998; Shao et al., 2000; Twaddle et al., 1999). The concentration lower than 10 micromol/L, the growth of MCF-7 cell, an estrogen receptor positive cancer cell line, was stimulated by genistein, however, genistein does not stimulate the growth of estrogen receptor-negative breast cancer cell. In vivo study also demonstrated dose-dependency of genistein. In MCF-7 cell implanted athymic, ovariectomized mice, genistein, at the concentration of 750 microg/g, stimulated tumor cell growth. The concentration of genistein under 300 microg/g was not as much as stimulatory (Allred et al., 2001). The biphasic effect has been attributed to the genistein exerting estrogen-like effects at lower concentrations, but at higher levels, genistein might act as an estrogen- antagonist. The estrogen binding experiment using chimeric proteins of ER alpha and beta binding domain has shown that genistein is ER ligand and stimulates the proliferation of ER alpha dependent breast cancer cell lines, and the cytotoxicity of genistein at a higher concentrations was ER independent (Maggiolini et al., 2001). Genistein has been revealed to arrest cell cycle at the G2/M phases in breast cancer cells, prostate cancer cells, gastric cancer cells and lung cancer cells. Shao et al. (1998) have found that genistein has an inhibitory effect on cell proliferation both in ER- positive and ER-negative human breast cancer cells. This inhibitory effect was attributed to G2/M arrest and accompanied by increased p21 expression, mediated by stabilizing effects on p21 through p53 independent mechanism.

Davis et al. (1998) has also shown that induction of apoptosis by genistein is accompanied by G2/M arrest and up-regulation of p21 and down-regulation of cyclin B in prostate carcinoma cells. In other studies, the increase in p21 were found to be correlated with either G2/M arrest (Lian et al., 1998) or G1/S block (Kuzumake et al., 1998; Shen et al., 2000). However, in human melanoma cells, upregulation of p21 did not appear to be required for G2/M arrest (Casagrande et al., 2000, Casagrande et al., 2001).

Apoptotic cell death accompanied by cell cycle delay in the G2/M phase has been observed with many cancer cell lines treated with genistein (Traganos et al., 1992, Matsukawa et al., 1993, Li et al., 1999, Lian et al., 1999). The possible regulation of bcl-2 and/or bax has been the focus of apoptotic cell death mechanism, and the results were not consistent. In genistein-treated MCF-7 cells, bcl-2 phosphorylation was increased 24-48 post-treatment and bax expression was not increased during this time (Constatinou et al., 1998). Li et al. (1999) have found that genistein induced up-regulation of bax, proapoptotic

protein and down-regulation of bcl-2, which protects cells from programmed death be forming heterodimers with bax and reducing the number of bax homodimers. The concentration dependency of bcl-2 expression has been observed with MCF-7 cells (Leung et al., 2000). At the threshhold concentration of genistein inducing apoptosis (25 micromol/L), bcl-2 expression was not elevated however, at the higher concentrations both of bcl-2 and bax expressions were increased. MCF-7 cells were differed in their sensitivity to apoptosis compared to the MDA-MB-231 cells with genistein, and bax: bcl-2 ratio was initially increased but later decreased after 48 hr (Xu and Loo, 2001). The involvement of caspase-3 protease in mediating genistein-induced apoptotic pathway has been suggested by the experiment with prostatic carcinoma cell lines by the study of Kumi-Diaka et al. (2000).

PKCs are known to be involved in the induction of COX-2 expression (Blanco, et al., 1995). Genistein, known PKC inhibitors was shown to be a potent inhibitor of transcriptional activation of COX-2 in macrophages (Liang et al., 1999). Using beta-galactosidase reporter gene system in human colon cancer cells, Mutoh et al. (2000) have shown that genistein suppress the COX-2 promoter activity with and without TNFalpha-stimulation.

In this study, the effects of genistein and estrogen or in the combination of genistein and estrogen on cell regulatory protein expressions in MCF-7 cells were compared, and it was found that genistein has estrogen agonistic and antagonistic activity depending on concentrations. At the concentrations of 25 microM and 50 microM, p21 and p53 expressions were similar to those of estrogen. However, at the higher concentration of 100 microM genistein had antiestrogenic activity increasing expression of these proteins in the presence of estrogen. Estrogen has been suggested as a promoter of breast cancer for its cell proliferative effect (Nenci et al., 1988) and an antiapoptotic agent (Perillo et al., 2000). Genistein could induce apoptosis at 50 and 100 microM and estrogen could tamper apoptotic activity of genistein at 50 microM as shown with the DNA fragmentation assay.

Antiapoptotic activity of estrogen has been attributed to the increment of cyclin B_1 by estrogen in MCF-7 cells (Zoubine et al., 1999). In this study, the elevated cyclin B_1 levels by estrogen was lowered by co-treatment with genistein (100 microM), suggesting that antiapoptotic cell regulation by estrogen could involve cyclin B_1 and genistein interacts with estrogen in estrogen antagonistic pattern at the higher concentration. The balance between proapoptotic bax and antiapoptotic bcl_2 appeared to play a role in susceptibility to apoptosis (Minn et al., 1998). Genistein increased the expression of bax in a dose-dependent manner, and estrogen decreased bax protein depending on the concentrations. The co-treatment of genistein and estrogen showed synergistic activity in the three different concentrations. At the concentrations tested both genistein and estrogen did not appear to influence bcl_2 expression in this study. In estrogen-deficient female rats, the two different concentrations of genistein were employed along with estrogen treatment. Estrogen agonistic activity was shown with the higher concentrations of genistein in bax expression. In whole animal tissues, p21, pp53 and bcl_2 expression did not seem to be related to bax expression with low and high concentrations of genistein.

Cyclooxygenase (COX) metabolizes arachidonic acid to prostaglandins and thromboxanes. Among the two isoforms of COX, COX-2 is the enzyme induced by proinflammatory stimuli, cytokines, and mitogens. Evidence is now emerging to show that COX-2 is involved in tumorigenesis (Subbaaramaiah et al., 1996). There is a possibility that COX-2 is involved in genistein-induced apoptosis. In whole animal tissue, the lower concentration of genistein showed estrogen agonistic activity in COX-2 expression. The examination of the role of COX-2 in genistein-induced apoptosis can provide the infor-

mation that COX-2 is the molecular target of tumorigenesis through apoptosis related mechanism.

This study indicates that genistein has a potential to inhibit the proliferation of tumor cells through apoptosis mechanism. However, the observed agonistic or antagonistic activity of genistein to estrogen *in vitro* and *in vivo* systems with a concentration dependent trend requires further investigation to test the dose of genistein in the prevention of various cancers.

REFERENCES

1) Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hamalainen E, Hasegawa T, Okada H. Urinary excretion of lignins and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional diet. *Am J Clin Nutr* 1991; 54: 1093-1100.
2) Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. Genistien, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 1987; 262: 5592-5595.
3) Allred CD, Allred KF, Virant SM, Helferich WG. Soy diets containing varying amounts of genistein stimulate growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner. *Cancer Res* 2001; 61: 5045-5050.
4) Blanco A, Habib A, Levy-Toledano S, Maclouf J. Involvement of tyrosine kinases in the induction of cyclo-oxygenase in human endothelial cells. *Biochem J* 1995; 312: 419-423.
5) Brzozowski AM, Pike CW, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson J, Carlquist M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 1997; 389: 753-758.
6) Casagrande F, Darbon JM. $P21^{CIP1}$ is dispensable for the G2 arrest caused by genistein in human melanoma cells. *Exp Cell Research* 2001; 258: 101-108.
7) Casagrande F, Darbon JM. Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cylcin-dependent kinases CDK2 and CDK1. *Biochem Pharmacol* 2001; 61: 1205-1215.
8) Constatinou AI, Kamath N, Murley JS. Genistein inactivates bcl-2, delays the G2/M phase of the cell cycle, and induces apoptosis of human breast adenocarcinoma MCF-7 cells. *Eur J Cancer* 1998; 34: 1927-1934.
9) Davis JN, Singh B, Bhuiyan M, Sarkar FH. Genistein-induced upregulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. *Nutr Cancer* 1998; 32: 123-131.
10) Goodman MT, Wilkens LR, Hankin JH, Hyu LC, Wu AH, Kolonel LN. Association of soy and fiber consumption with the risk of endometrial cancer. *Am J Epidemiol* 1997; 146: 294-306.
11) Herbert J, Hurley T, Olendzki B, Teas J, Yunsheng M, et al. Nutritional and socioeconomic factors in relation to prostate cancer mortality: a cross-national study. *J Nat Cancer Inst* 1998; 90: 1637-1647.
12) Hsieh CY, Santell RC, Haslam SZ, Helferich WG. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. *Cancer Res* 1998; 58: 3833-3838.
13) Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-oestrogens and breast cancer. *Lancet* 1997; 350: 990-994.
14) Kumi-Kiaka J, Sanderson NA, Hall A. The mediating role of caspase-3 protease in the intracellular mechanism of genistein-induced apoptosis in human prostatic carcinoma cell lines, DU 145 and LNCaP. *Biol Cell* 2001; 92: 595-604.
15) Kuzumake T, Kobayashi T, Ishikawa K. Genistein induces $p21^{CIP1/WAF1}$ expression and blocks the G1 to S phase transition in mouse fibroblast and melanoma cells. *Biochem Biophys Res Comm* 1998; 251: 291-295.
16) Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, Day NE. Dietary effects on breast cancer risk in Singapore. *Lancet* 1991; 336: 1197-1200.
17) Leung LK, Wang, TT.Bcl-2 is not reduced in the death of MCF-7 cells at low genistein concentration. *J Nutr* 2000; 130: 2922-2926.
18) Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. Induc-

tion of apoptosis in breast cancer cells MDA-MB-231 by genistein. *Oncogene* 1999; 18: 3166-3172.
19) Lian F, Bhuiyan M, Li YW, Wall N, Sarkar FH. Geinstein-induced G2-M arrest, $p21^{WAF1}$ upregulation, and apoptosis in a non-small-cell lung cancer cell line. *Nutr Cancer* 31, 184-191.
20) Lian F, Li Y, Bhuiyan M, Sarkar FH. p53-independent apoptosis induced by genistein in lung cancer cells. *Nutr Cancer* 1999; 33: 125-131.
21) Liang Y, Huang Y, Tsai S, Lin-Shiau S, Chen C, Lin J. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis* 1999; 20: 1945-1952.
22) Liang YC, Lin-Shiau SY, Chen CF, Lin JK. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (-)-epigallocatechin-3-gallate. *J Cell Biochem* 1999; 75: 1-12.
23) Maggiolini M, Bonofiglio D, Marsico S, Panno M.L, Cenni B, Picard D, Ando S. Estrogen receptor alpha mediates the proliferative but not the cytotoxic dose-dependent effects of two major phytoestrogens on human breast cancer cells. *Mol Pharmacol* 2001; 60: 595-602.
24) Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* 1978; 103: 1860-1867.
25) Matsukawa Y, Marui N, Sakae T, et al. Genistein arrests cell cycle progression at G2-M. *Cancer Res* 1993; 53: 1328-1331.
26) Minn AJ, Swain RE, Ma A, Thompson CB. Recent progress on the regulation of apoptosis by bcl-2 family members. *Adv Immunol* 1998; 70: 245-279.
27) Mutoh M, Takahashi M, Fukuda K, Matsushima-Hibiya Y, Mutoh H, Sugimura T, Wakabayashi K. Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. *Carcinogenesis* 2000; 21: 959-963.
28) Nenci I, Marchetti E, Querzoli P. Commentary on human mammary preneoplasia. The estrogen receptor-promotion hypothesis. *J Steriod Biochem* 1988; 30: 105-106.
29) Pagliacci MC, Smacchia M, Migliorati G, Grignani F, Riccardi C, Nicoletti I. Growth- inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells. *Eur J Cancer* 1994; 11: 1675-1682.
30) Perillo B, Sasso A, abbondanza C, Palumbo G. 17 beta-estradiol inhibits apoptosis in MCF-7 cells, including bcl-2 expression via two estrogen-responsive elements present in the coding sequence. *Mol Cell Biol* 2000; 20: 2890-2901.
31) Peterson G, Barnes S. Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. *Biochem Biophys Res Comm* 1991; 19: 661-667.
32) Peterson G, Barnes S. Genistein inhibits both estrogen and growth factor-stimulated proliferation of human breast cancer cells. *Cell Growth Differ* 1996; 7: 1345-1351.
33) Shao A, Alpaugh ML, Fontana JA, Barsky SH. Geinstein inhibits proliferation similarly in estrogen receptor-positive and negative human breast carcinoma cell lines characterized by $p21^{WAF1/CIP1}$ induction, G_2/M arrest, and apoptosis. *J Cell Biochem* 1998; 69: 44-54.
34) Shao ZM, Shen ZZ, Fontana JA, Barsky SH. Genistein's ER-dependent and independent actions are mediated through ER pathways in ER-positive breast carcinoma cell lines. *Anticancer Res* 2000; 20: 2409-2416.
35) Shao ZM, Wu J, Shen ZZ, Barsky SH. Genistein exerts multiple suppressive effects on human breast carcinoma cell. *Cancer Res* 1998; 58: 4851-4857.
36) Shen J, Klein RD, Wei Q, Guan Y, Contois JH, Wang TTY, Chang S, Hursting SD. Low-dose genistein induces cyclin-dependent kinase inhibitors and G_1 cell-cycle arrest in human prostate cancer cells. *Mol Carcinogenesis* 2000; 29: 92-102.
37) Shutt DA, Cox RI. Steroid and phytoestrogen binding to sheep uterine receptors in vitro. *J Endocrinol* 1972; 52: 299-310.
38) Subbaramaiah K, Telang N, Ramonetti JT, Araki R, DeVito B, Weksler BB. Transcription of cyclooyxgenase-2 is enhanced in transformed mammary epitheilial cells. *Cancer Res* 1996; 56: 4424-4429.

39) Traganos F, Ardelt B, Halko N, Bruno S, Darzynkiewicz Z. Effects of genistein on the growth and cell cycle progression of normal human lymphocytes and human leukemic MOLT-4 and HL-60 cells. *Cancer Res* 1992; 52: 6200-6208.
40) Twaddle GM, Turbov J, Liu N, Murthy S. Tyrosine kinase inhibitors as antiproliferative agents against an estrogen-dependent breast cancer cell line *in vitro*. *J Surg Oncol* 1999; 70: 83-90.
41) Xu J, Loo G. Different effects of genistein on molecular markers related to apoptosis in two phenotypically dissimilar breast cancer cell lines. *J Cell Biochem* 2001; 82: 78-88.
42) Zheng W, Dai Q, Custer L, Shu XO, Wen WQ, Jin F, Franke AA. Urinary excretion of isoflavonoids and the risk of breast cancer. Cancer Epidemiol. *Biomark Prev* 1999; 8: 35-40.
43) Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian- American women. *J Nat Cancer Inst* 1993; 85: 1819-1827.
44) Zoubine MN, Weston AP, Johnson DC, Campbell, DR, Banerjee SK. 2-Methoxyestradiol-induced growth suppression and lethality in estrogen-responsive MCF-7 cells may be mediated by down regulation of p34cdc2 and cyclin B1 expression. *Int J Oncol* 1999; 4: 639-646.