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TITLE: Molecular Mechanism of Action of Genistein and Related Phytoestrogens in Estrogen Receptor Dependent & Independent Growth of Breast Cancer Cells

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ABSTRACT.

During the first year of my pre-doctoral fellowship, I have demonstrated that genistein binds weakly to the estrogen receptor (ER) with an IC_{50} value of 900 nM (task 1). Quercetin and genistein also bind to the type-II estrogen binding sites, and exert cell growth inhibition in MCF-7 and MDA-MB-468 cells (task 2). In circular dichroism studies, decreased intensity of the spectra leading to a random recombinant ER structure was observed with genistein (task 3). Genistein (10 μM) stimulated the growth of MCF-7 cells at 24 h, however, by 72 h, it was growth inhibitory at all doses (task 5). A major cell cycle block at G_2/M phase was observed with genistein treatment and was accompanied by alterations in cyclin B1 levels (tasks 7 and 8). In addition, we show that cell growth inhibition by genistein and quercetin is associated with decreased polyamine levels. Our results provide insights into the ER-dependent and -independent mechanisms of action of genistein in breast cancer cell growth.
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Srivani B
PI - Signature

6-30-98
Date
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A. INTRODUCTION

The primary focus of my research in the past year had been to understand the complex behavior of the phytoestrogen genistein in promoting or inhibiting the growth of human breast cancer cells.

Epidemiological studies suggest that Oriental women consuming high vegetarian diets are at a lower risk for breast cancer (1,2,3). The anticancer effects of the vegetarian diets are attributed to the phytoestrogens (flavones, isoflavones, coumestans and lignans), present abundantly in soy products, fruits, vegetables, and whole-grains (4,5,6). Hormone-dependent cancers such as breast cancer is promoted by endogenous estradiol (7). Estradiol (E2) binds the estrogen receptor (ER), the complex then binds estrogen response element (ERE) and increases the transcription of growth-related genes (8). Owing to the structural similarity to estradiol, and potential antiestrogenic effects (9,10), phytoestrogens are being studied for their potential use in chemoprevention.

Genistein is an isoflavone that has been shown to have both stimulatory (11,12) as well as inhibitory roles (13,14,15) in cancers in humans and animals. In breast cancer, the growth enhancing effects of genistein are attributed to its estrogen-like activity (16,17), while its antiproliferative actions were explained either as antiestrogenic (18) or as estrogen-independent (19,20). This study is a systematic evaluation of the dose-dependent estrogenic, antiestrogenic or ER-independent pathways through which genistein exerts its diverse effects on breast cancer cells.

We explored the mechanism of ER-based estrogenic actions of genistein by determining the binding affinities of genistein to ER using cellular and recombinant ER (rER). To understand how the molecular structure of genistein is associated with estrogenic or antiestrogenic properties, we compared it with other structurally related compounds such as quercetin, daidzein, biochanin A and kaempferol in ER binding. To further delineate the ER-mediated actions of genistein, we conducted circular dichroism (CD) studies. These data are expected to provide evidence for any secondary structural changes in ER protein after its binding to genistein. The changes in spectral intensity caused by estradiol are compared to that obtained with different concentrations of genistein.

Estradiol also binds to another class of protein besides ER, called the type II binding site (EBS) with low affinity (21). Reported evidence suggests that the function of EBS may not be to bind physiological concentrations of estradiol (22). However, there is some evidence indicating that plant flavonoids may be the ligands for EBS and mediate cell growth inhibition by these compounds (21). To explore this possibility for phytoestrogens’ action independent of ER, we quantified EBS and determined their affinity for phytoestrogens relative to estradiol in ER-positive MCF-7 and ER-negative MDA-MB-468 breast cancer cells.

Cell proliferation is strictly controlled in normal cells whereas in cancer cells, a strict restriction point control is lacking, so they continue to grow under conditions in which normal cells are dormant (23). Recently, considerable interest has been paid to the role of cyclins in regulating cell cycle progression, and these proteins may be a target for
chemopreventive agents (24). To comprehend the mechanism of action of genistein and its structural basis in mediating growth inhibition, we studied the cell cycle distribution after treatment with genistein and other structurally similar phytoestrogens in breast cancer cells. To further identify the targets of this molecule in cancer cells, we determined the levels of cyclin B1 protein. Cyclin B1 is needed for the successful progression of cells from S to M phase and for the completion of mitosis (25).

Polyamines are cellular cations, present abundantly in all cells (26). They are involved in cell growth and differentiation (26). Previous studies reported that estradiol based growth in breast cancer cells is mediated through an increase in intracellular polyamine levels (26,27,28). Ornithine decarboxylase (ODC) is the first and a rate limiting enzyme in the synthesis of cellular polyamines (26). To gain insight into the estrogenic or antiestrogenic actions of genistein, we determined ODC activity and polyamine levels after treatment of breast cancer cells with this compound.

Our results in the first year provide some important clues to the mechanism of action of genistein in breast cancer cells. In addition to genistein, we studied other structurally related compounds. The chemical structures of the phytoestrogens used in this study are shown in Figure 1.

A summary of my accomplishments during this report period is given below.

<table>
<thead>
<tr>
<th>Tasks for this report</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>1. Months 1-3</td>
<td>Binding affinities of phytoestrogens with cellular and recombinant ER- <strong>completed</strong></td>
</tr>
<tr>
<td>2. Months 4-6</td>
<td>Binding affinities of phytoestrogens with type-II EBS - <strong>completed</strong></td>
</tr>
<tr>
<td>3. Months 7-12</td>
<td>Molecular conformation of phytoestrogen-bound ER- <strong>Partially completed, work in progress.</strong></td>
</tr>
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Tasks proposed for the second and third years

5. Months 17-19 | Cell growth studies - **completed**
7. Months 27-28 | Cell cycle progression studies- **completed**
9 Months 29-36 | Cyclin levels determination - partially **completed**
* Additional tasks | Measurement of polyamine levels - **completed**
| Determination of ODC activity - partially **completed**

*These experiments were not proposed, but provide additional information on the estrogenic or antiestrogenic actions of genistein.

Presentations
2. Gave an oral presentation titled “Genistein treatment causes G2/M cell cycle arrest and cyclin B1 accumulation in breast cancer cells: a possible mechanism for the preventive action of genistein”, in the focus session during the Annual Retreat on Cancer

Award. Received the “Gallo Award”, presented by the Cancer Institute of New Jersey for “Outstanding Cancer Research”, May 29, 1998 (COPY ATTACHED).

A brief description of methods used, results and discussion follows.

B. METHODS

1. Cell culture. MCF-7 cells are maintained in Dulbecco's modified Eagle's medium (DMEM) with 100 μg/ml penicillin, 100 μg/ml streptomycin, 2 μg/ml insulin, and 10% fetal bovine serum (FBS). For two weeks before each experiment, cells were grown in phenol red free DMEM as phenol red has estrogenic effects (29). FBS was treated with dextran coated charcoal (DCC) to remove endogenous estrogens and added to the medium as described previously (30). MDA-MB-468 cells were grown in Improved minimum essential medium with 5% FBS, 2 mM glutamine, and 100 μg/ml each of penicillin and streptomycin.

Genistein, quercetin, daidzein, kaempferol were purchased from Sigma Chemical Co. (St Louis, MO). Stock solutions were made in dimethyl sulfoxide (DMSO), aliquoted and frozen until use. Recombinant ER was purchased from Panvera Corp (Madison, WI).

2. Competitive binding assay of phytoestrogens with labeled estradiol. To prepare cellular ER, MCF-7 cells from 10 T-150 flasks were harvested into PBS and sonicated in TEDG buffer (10 mM Tris, 1 mM EDTA, (pH 7.5), 1 mM dithiothreitol (DTT) and 10% glycerol). The cellular homogenate was centrifuged at 100,000 x g to obtain the cytosol. The cytosol (250 μl) was incubated for 3 h at 4 °C with 5 nM [3H]-E2 and unlabeled genistein or quercetin (10^-5 - 10^-8 M). After incubation, DCC suspension was added to the samples to remove free E2. The samples were then centrifuged at 750 x g for 5 min at 4 °C. Supernatant was sedimented through a 10-30% sucrose-TEDG buffer. [14C]-labeled bovine serum albumin and γ- globulin were used as internal markers. Gradients were centrifuged in a Beckman SW60 rotor at 53,000 rpm for 16 h. Fractions were collected into 5 ml scintillation fluid and the bound estradiol was counted using a scintillation counter. Nonspecific binding was determined in parallel samples incubated with a 200-fold molar excess of unlabeled estradiol. Concentration dependent, competitive binding curves were determined for each compound. Results are expressed as percent [3H]-E2 bound in the absence (100%) or presence of the competitor.

Human recombinant ER (500 fmols/ml) was incubated with 5 nM [3H]-E2 and different concentrations of the phytoestrogens, and treated as described above for cellular ER. In parallel experiments, receptor was incubated with 5 nM [3H]-E2 and increasing concentrations of unlabeled E2.
3. **Type-II EBS analysis in human breast cancer cells.** Type-II EBS were measured by a whole cell assay as described by Ranelletti et al (31). MCF-7 and MDA-MB-468 cells (5 x 10^4/well) were plated into Multiwell plates with the appropriate medium. After 24 h, the cells were refed with fresh media without serum. The cells were then dosed with increasing concentrations (4-50 nM) of [3H]-estradiol alone or with 200-fold molar excess of diethyl stilbestrol (DES) and incubated for 3 h at 37 °C. After 3h, the cells were kept on ice for 30 min and washed 4 times in 1 ml volumes of ice cold HBSS. To each well, 1 ml of 100% ethanol was added and incubated at 37 °C. Bound estradiol was measured by a liquid scintillation counter. Competition for type-II EBS was determined by incubating cells with 40 nM [3H]-E2 plus increasing concentrations of one of the phytoestrogens. Radioactivity bound to the cells was determined and IC50 values were calculated for each of the compounds, from the concentration dependence of the displacement of [3H]-E2 from type-II EBS.

4. **Circular dichroism measurements.** The secondary structural changes in the human recombinant ER (1.5 μM protein) was studied in the presence of genistein, using CD spectroscopy. Genistein (0.5-10 μM) or same concentrations of estradiol were incubated with the ER at room temperature overnight in storage buffer (50 mM Tris, 1 mM sodium vanadate, 1 mM EDTA, 500 mM KCl, 10% glycerol and 2 mM DTT). All CD spectra were recorded using an AVIV 62D spectropolarimeter with a water-jacketed cell of 1 mm path length. The spectral wavelength range measured was between 200-260 nm. The cell temperature was kept constant with a circulating water bath at 25 °C. The α-helicity was calculated from the reported value of molar ellipticity of a 100% helical peptide, (θ) (222 nm) = 33,500 deg cm^2 dmol^-1.

5. [3H]-thymidine incorporation assay. Cells (0.5 x 10^6) were plated in 60 mm culture dishes and allowed to adhere for 24 h. The cells were dosed with different concentrations of phytoestrogens for 24, 48 and 72 h. One hour before the treatment time ended, one μCi/ml of [3H]-thymidine was added to the cells. The radioactivity incorporated into the cellular DNA was measured by liquid scintillation counting.

6. **Flow cytometry.** To determine the effect of phytoestrogens on cell cycle progression, flow cytometric analysis was conducted. We plated 2 x 10^6 cells in 100 mm dishes. At 24, 48 and 72 h after the treatment of cells with different concentrations of phytoestrogens, the media was removed and 2 ml of the buffer containing 40 mM sodium citrate, 250 mM sucrose, and 5% DMSO was added. The samples were then frozen at -70 °C until further analysis. For DNA analysis, the cells were thawed, citrate buffer removed, and trypsinized for 10 min on ice. Then a solution of trypsin inhibitor and RNase were added for 10 min and stained with propidium iodide in citrate buffer (130 μg/ml). Cells were analyzed by a Coulter Flow Cytometer. Distribution of cells in different phases of cell cycle was calculated using cytological software.
7. Western blot analysis of cyclin B1. Cells were plated as described above for Flow cytometry. Twenty four hours after treatment with genistein and quercetin, cells were harvested in PBS and pellets stored at -70 °C until further analysis. Cell pellets were solubilized in 300 μl of a buffer containing 150 mM Tris, 150 mM NaCl, 50 mM NaF, 0.2% SDS, 1% NP40, 2 mM EDTA, 100 μM Na3PO4. Thirty μg protein was electrophoretically separated on a 10% polyacrylamide gel. The proteins were transferred to PVDF immobilon membrane. After blocking overnight with 2% non-fat dry milk, blots were incubated for 3 h with purified monoclonal mouse anticyclin B1 (NeoMarkers, CA) antibody, followed by horseradish-peroxidase labeled anti-mouse secondary antibody. Protein was visualized with a chemiluminiscence based detection system and the intensity of the bands were read by a scanning densitometer.

8. Determination of ODC activity. MCF-7 cells were harvested after treatment with genistein. The cell pellet was resuspended in 300 μl cold assay buffer and stored at -70 °C. Thawed samples were sonicated and centrifuged at 12,000 x g for 10 min at 4 °C. To 100 μl of the supernatant, 200 μl of assay buffer and 0.08 mM pyridoxal phosphate (final) was added. The reaction was initiated by adding 100 μl L-ornithine and 0.25 μCi of L-[1-14C]-ornithine to yield a final concentration of 0.5 mM ornithine. The released 14CO2 was absorbed on a GF/C filter paper during incubation and was measured using the liquid scintillation counter. The amount of protein in cell lysates was determined and ODC activity expressed as pmole/mg protein/min.

9. Determination of intracellular polyamine levels. Cells (2 x 10^6) were plated and after 24, 48 and 72 h treatment with genistein and quercetin, were harvested in 1 x PBS. The cell pellet was treated with 300 μl of 8% sulfosalicylic acid, and sonicated for 15 sec on ice. The lysate was incubated on ice for 1 h and centrifuged at 10,000 x g for 5 min in a microfuge tube to remove the precipitated protein. Polyamine levels were determined by high performance liquid chromatography (HPLC) after derivatization to their dansyl derivatives. 1,6-diaminohexane was used as an internal standard.

C. RESULTS

I. Results of Task I: Binding affinities of phytoestrogens with ER, months 1-3.

To determine if phytoestrogens interact with the ER, we studied their binding with ER, relative to unlabeled estradiol using sucrose gradient analysis. Recombinant as well as cellular ER prepared from MCF-7 cells were used in the experiments. The inhibition of [3H]-E2 binding to cellular and recombinant ER by genistein is shown in Figures 2 and 3, respectively. The IC50 value, the concentration of phytoestrogens needed to displace 50% of the [3H]-E2 bound to cellular ER or recombinant ER is given in Table 1. The order of relative affinities of the phytoestrogens for ER as compared to estradiol are as follows; genistein >> quercetin > daidzein ≥ biochanin A ≥ kaempferol. The IC50 values of different compounds showed comparable rank order with cellular and recombinant ER.
However, [³H]-E₂ from recombinant ER is more easily displaced by phytoestrogens than cellular ER.

II. Results of Task 2: Interaction of phytoestrogens with type-II EBS, months 4-6.

In order to investigate the ER-independent actions of phytoestrogens, we measured relative binding affinities of phytoestrogens for type-II EBS. First, we determined type-II EBS in MCF-7 and MDA-MB-468 cells according to the whole-cell assay (31). In the ER-positive MCF-7 cells, the specific binding of [³H]-E₂ was determined by competing out with DES. Two classes of specific binding sites were observed, one between 5 and 10 nM and another at 40 nM concentration of radiolabeled E₂ (data not shown). These sites correspond to the ER (saturating at 5-10 nM concentrations) and type-II EBS (showing saturation at 40 nM concentration), as described in a previous study (32). Further experiments to determine competition of genistein for type-II EBS were conducted using 40 nM [³H]-E₂ in MCF-7 cells. In MDA-MB-468 cells, 30 nM [³H]-E₂ saturated the type-II EBS, and this concentration was used to determine competition by phytoestrogens.

Among the phytoestrogens studied, quercetin bound to the type-II EBS with high affinity, with an IC₅₀ of 35 nM and 590 nM in MDA-MB-468 and MCF-7 cells, respectively (Figure 4, Table 2). Genistein, however, was less potent requiring >2 μM concentrations to inhibit [³H]-E₂ binding in both the cell lines (Table 2). Kaempferol and daidzein did not compete for type-II EBS with [³H]-E₂, up to 10 μM concentration.

III. Results of Task 3: Changes in molecular conformation of phytoestrogen-bound ER using CD studies, months 7-12.

To gain insight into the conformational alterations caused by genistein on ER, we conducted CD studies using recombinant human ER. The recombinant ER was incubated overnight at room temperature in storage buffer, to eliminate any time dependent changes in the protein during analysis with the compounds. Increasing concentrations of genistein were added to the rER and CD spectra recorded at 25 °C. The results are presented in Figure 5. The CD spectrum of ER alone showed a minima spectra at 225 nm and a shoulder band at 212 nm. Genistein and estradiol decreased the intensity of the CD spectra. Calculation of the percentage of α-helix content was made from the multiple linear regression program developed by Yang et al (33), which gave an estimated α-helix contents of 26.9 % for untreated ER, 17.5% and 13.5% with 10 μM estradiol and genistein, respectively. These results are however, in a preliminary stage and more experiments will be performed to understand the ligand dependent changes in the ER.

In addition to the three tasks required to be completed in the first year, I also worked on other tasks proposed for second and third years of my fellowship. Results from these experiments are presented below.
IV. Results of Task 5. Cell proliferation studies, months 17-19.

In order to relate binding affinity data to biological effects of phytoestrogens, we determined the effects of these compounds on cell growth. MCF-7 and MDA-MB-468 cells were treated with 0, 10, 25, 50 and 100 μM phytoestrogens for 24, 48 and 72h. Cell growth was determined by [3H]-thymidine incorporation assay. In MCF-7 cells, 10 and 25 μM genistein treatment for 24 h caused a 27% and 39% increase in cell growth, respectively. However, the higher concentrations (50 and 100 μM) were growth inhibitory. Furthermore, by 72 h of treatment, all doses of genistein were growth inhibitory (Figure 6). Genistein significantly reduced the growth of MDA-MB-468 cells at all concentrations used and the concentration needed to decrease the growth of ER-negative cells was 4-fold lower than that needed for the ER-positive cells. The IC50 values for the phytoestrogens in inhibiting the growth of MCF-7 and MDA-MB-468 cells are presented in Table 3. Quercetin, although less effective than genistein, was equally potent in inhibiting cell growth of ER-positive and -negative cells. Biochanin A and kaempferol were less potent cell growth inhibitors where as daidzein did not have a significant effect in decreasing the growth of these breast cancer cells at the concentrations used.

VI. Results of Task 7. Cell cycle progression after treatment with phytoestrogens, months 27-28.

Flow cytometric analysis of the untreated breast cancer group showed a majority of the cells in the G0/G1 (65-70%) phase, and only a small percentage of cells in the G2/M phase (10-13%) of the cell cycle in both MCF-7 and MDA-MB-468 cells. We observed a time and dose-dependent accumulation of the G2/M phase cells after treatment with genistein in MDA-MB-468 cells (Figure 7). The increase in the population of G2/M cells was associated with a corresponding decrease in the percentage of cells in S and G0/G1 phases of the cell cycle. Genistein treatment (100 μM) for 24 h caused 70% of the MDA-MB-468 cells and 42.4% of the MCF-7 cells to arrest at the G2/M phase (Table 4 and 5 respectively). Quercetin treatment also caused G2/M block, although to a lesser extent compared to genistein. The effects of G2/M block by genistein and quercetin were much more prominent in MDA-MB-468 cells. Kaempferol was half as effective as quercetin, while biochanin A and daidzein did not have significant effects on cell cycle distribution in MDA-MB-468 cells.

VII. Results of Task 8. Western Immunoblot for cyclin protein determination, months 29-36.

Genistein and quercetin caused a significant G2/M block in the breast cancer cells. Therefore, further studies were conducted to study the effects of phytoestrogens on the levels of cyclin B1 as it is involved in G2/M progression. Treatment of the breast cancer cells with genistein resulted in a biphasic response in cyclin B1 levels. After 24 h of treatment, 50 μM genistein caused an accumulation of cyclin B1 protein compared to control in MCF-7 cells (Figure 8A) and 25 μM genistein increased cyclin B1 levels in
MDA-MB-468 cells (Figure 9). However, at 100 µM concentration, genistein caused a drastic decrease in the levels of this protein in both the cell lines.

In contrast to genistein, quercetin-treated MDA-MB-468 cells showed a rise in cyclin B1 levels (Figure 9) amounting to a 37% increase with 100 µM treatment compared to controls as determined by densitometric scanning. Similarly, cells treated with the positive control nocodazole (known microtubule inhibitor) (34) resulted in a progressive accumulation of cyclin B1 levels after 24 h (Figure 8B).

VIII. Results of additional experiments. In addition to the studies proposed, we conducted other experiments which enhanced our understanding of estrogen-dependent and independent actions of genistein in breast cancer cell growth.

Effect of genistein treatment on ODC activity. The polyamine biosynthetic enzyme ODC activity increased significantly within 4 h of genistein treatment (Figure 10A). However, at later time points, the enzyme activity was greatly decreased in MCF-7 cells (Figure 10 B).

Effect of genistein on intracellular polyamine levels. Genistein treatment for 24 h did not have a significant effect on the levels of putrescine, spermidine and spermine in MCF-7 cells. However, at 48 h and 72 h, there was a decrease in all the polyamine levels (Figure 11). Similar results were obtained with quercetin treatment (data not shown).

D. DISCUSSION

In this report, we present data on the effects of phytoestrogens on a number of parameters likely to be important in the biological action of these compounds. Competitive binding of phytoestrogens to cellular and recombinant ER shows genistein to have higher binding affinity to ER compared to other structurally related compounds, quercetin, daidzein, biochanin A and kaempferol. Assessment of type-II EBS in MCF-7 and MDA-MB-468 cells revealed a relatively higher binding affinity of quercetin to these sites compared to other compounds. Studies on cell growth inhibition showed quercetin to be the most active compound in MCF-7 cells with IC$_{50}$ of 17.3 ± 2.7 µM, where as genistein is the most active compound in MDA-MB-468 cells with an IC$_{50}$ of 8.8 ± 1.6 µM. Thus, even though genistein has relatively higher binding affinity for ER, compared to other compounds, this affinity does not seem to be important in its antiproliferative effects. Our studies on cell cycle kinetics showed genistein-induced arrest of MDA-MB-468 cells in the G2/M phase, and a biphasic response in cyclin B1 levels. These results will be potentially important in the identification of cellular targets of genistein, and phytoestrogens in general.

The concentration of genistein effective in displacing 50% of estradiol from ER was reported previously to be growth stimulatory in breast cancer cells (35). Therefore, it has been a great concern that at physiologically relevant concentrations (1 nM - 10 µM), genistein stimulates ER-positive breast cancer cells via an estrogenic response (35). In
our study, although genistein, daidzein, biochanin A and kaempferol at 10 μM stimulated MCF-7 cell growth in 24 h experiments, growth inhibition was evident by 3 days with genistein treatment. Also, we found the growth stimulation of 10 μM genistein only in the absence of estradiol and not when it is present (data not shown). These results are comparable to those reported by others (36,37). Similarly, Wang et al (17) reported that when MCF-7 cells were treated with 1 μM genistein for 6 days, ER mRNA was downregulated. Taken together, these results suggest that: (i) physiologically achievable concentrations of genistein may not be growth stimulatory in the presence of circulating E2 levels, and (ii) with chronic exposure, genistein may be working through other ER-independent mechanisms to inhibit the growth of breast cancer cells, even at low concentrations. In this study, quercetin bound weakly to the ER, but was growth inhibitory at all concentrations studied. These results are comparable to other studies, in which the quercetin concentration needed to bind the ER, was growth inhibitory (36).

Previous studies reported that flavonoids bind the type-II EBS and may cause a reduction in breast cancer cell growth. Quercetin was shown to induce type-II EBS in MDA-MB-231 cells (38) and this was associated with decreased cell growth. In our studies, quercetin bound to type-II EBS with higher affinity than other compounds, both in MCF-7 and MDA-MB-468 cells, as estimated from competition binding curves. The concentrations needed to half-saturate type-II EBS were 590 and 35 nM in MCF-7 and 468 cells respectively. However, binding affinity for genistein was lower than that of quercetin. Furthermore, biochanin A and kaempferol, which have very little affinity for ER had virtually no affinity for type-II EBS. Therefore, it is unlikely that type-II EBS play a significant role in the antiproliferative effects of most phytoestrogens.

We report for the first time, the concentrations of phytoestrogens needed to displace 50% of radiolabeled E2 bound to the recombinant ER. To understand the structural basis for ER-binding by genistein, we compared the effects of other structurally similar compounds in their ability to bind ER. Daidzein is similar in structure to genistein, except that it does not have the 5-OH group on its A ring (Figure 1). Biochanin A has the 5-OH group, but its 4'-OH group on B ring is methylated. Kaempferol differs from genistein in that its phenolic B ring is attached to the 2 position (instead of 3 position in genistein) of the pyran C ring and quercetin differs from kaempferol with an extra OH group on 5' position of the B ring (36). In a previous study, it was hypothesized that that the 4'-OH group of phenolic B ring and its spatial orientation with the 7-OH group on A ring are the reasons for genistein's estrogenicity (36,39). Therefore, when the 4'-OH group is methylated (biochanin A) or its orientation is changed (kaempferol), the binding affinity to ER is decreased (36,40), which is similar to what we observed. However, in our studies, daidzein, which is similar to genistein in this respect, has 10-fold lower binding with ER compared to genistein, suggesting that other factors beside the structure are important in ER binding.

Our results demonstrate that genistein decreased breast cancer cell growth, by arresting the proliferating cells in the G2/M phase of the cell cycle. The effects of genistein increased in a dose- and time- dependent manner in MDA-MB-468 cells. The G2/M block was associated with a decrease in the percentage of cells in the S and G0/G1
phases of the cell cycle. Similar to growth inhibition, the effects of genistein in increasing the population of G2/M cells were much more pronounced in the MDA-MB-468 cells compared to MCF-7 cells, suggesting that the estrogenic effects of genistein are not completely abated in MCF-7 cells. Our results confirm the previous reports of G2/M phase arrest by genistein in MCF-7 cells (41), and by quercetin in 468 cells (42). The inability of other phytoestrogens in this study to inhibit cell cycle progression at G2/M phase suggests that disruption of cell cycle is specific for quercetin and genistein.

In eukaryotic cells, cyclin B1 accumulates during the late S and G2 phases and allows entry of cells into the M phase and is rapidly degraded at the end of mitosis, allowing the cells to divide. In this study, genistein treatment resulted in the accumulation of cyclin B1 compared to untreated cells at 50 µM concentration in MCF-7 cells and at 25 and 50 µM concentration in MDA-MB-468 cells, suggesting that G2/M phase arrest is associated with the inhibition of cyclin B1 degradation. However, at 100 µM concentration, in both the cell lines, genistein treatment drastically decreased cyclin B1 levels after 24 h. Microscopic examination of the cells treated with highest dose of genistein showed morphological features of apoptosis, suggesting apoptosis as a reason for the observed decrease in cyclin B1 levels. In contrast to genistein, cyclin B1 levels increased after quercetin treatment for 24 h, similar to the microtubule inhibitor nocodazole. These results indicate that the mechanism of G2/M arrest by genistein and quercetin may be mediated via different pathways. It is unclear whether the observed alterations in cyclin B1 levels after treatment with genistein and quercetin are a result of a change in synthesis or degradation of this protein. Further studies will be directed towards answering this question.

Polyamine levels are increased several-fold in breast tumors compared to normal tissues (43), and have been reported to be involved in the estrogenic effects of cell growth (44). ODC, a key enzyme in the polyamine biosynthetic pathway, is transcriptionally regulated by estradiol and links the estrogenic effects and polyamine synthesis cascade. All the three polyamines, putrescine, spermidine and spermine levels decreased only after 48 and 72 h of genistein treatment in MCF-7 cells.

In conclusion, our results demonstrate that the antiproliferative effects of genistein are subdued in ER-positive breast cancer cells, because of its weak estrogenic potential. However, its role in cell growth inhibition involves several pathways, including disruption of cell cycle progression, alterations in cyclin B1 levels and disruption of polyamine pathway. Genistein induced alterations in different pathways followed similar trend in both ER-positive and negative cells leading to reduced cell growth, thus its antiproliferative effects are unlikely to be mediated by ER.
E. RECOMMENDATIONS IN RELATION TO THE STATEMENT OF WORK

I completed the first two tasks proposed for the first year of my grant period. Circular dichroism spectroscopy studies are in progress. In addition to the tasks in the first year, I completed most of the work from tasks 5, 7 and 9 proposed for subsequent years. I will be writing a manuscript soon with the data. I started some preliminary work in the polyamine pathway with the phytoestrogens, which will help broaden my understanding of the effects of phytoestrogens in breast cancer, and to elucidate the mechanism(s) of action of genistein in breast cancer cells.

F. CONCLUSIONS.

This study provides new information on the effects and mechanism of action of five phytoestrogens, genistein, quercetin, biochanin A, daidzein and kaempferol on breast cancer cell growth. All the phytoestrogens showed weak binding to the ER in MCF-7 cells compared to E2. Genistein is a potent breast cancer cell growth inhibitor in MCF-7 and MDA-MB-468 cells, and these effects appear to be independent of ER. In MDA-MB-468 cells, type-II EBS may have contributed to the antiproliferative effects of quercetin, because of relatively high affinity of quercetin for type-II EBS. However, since other compounds have low or no affinity for these sites, type-II EBS are unlikely to contribute to the effects of most phytoestrogens. We identified G2/M cell cycle arrest, disruption of cyclin B1 levels, and decrease in intracellular polyamine levels as potential pathways through which genistein and quercetin mediate their growth inhibition. Genistein and quercetin may have potential chemotherapeutic effects, and our results are valuable in understanding the targets of these drugs.
G. REFERENCES


<table>
<thead>
<tr>
<th>Phytoestrogen</th>
<th>IC₅₀ concentration, nM</th>
<th>MCF-7 ER</th>
<th>Recombinant ER</th>
</tr>
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<tr>
<td>Estradiol</td>
<td>5</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Genistein</td>
<td>900</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Quercetin</td>
<td>4000</td>
<td></td>
<td>880</td>
</tr>
<tr>
<td>Daidzein</td>
<td>8100</td>
<td></td>
<td>600</td>
</tr>
<tr>
<td>Biochanin A</td>
<td>8600</td>
<td></td>
<td>920</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>950</td>
<td></td>
<td>&gt; 1000</td>
</tr>
</tbody>
</table>

IC₅₀ values for the compounds were determined from the competition binding curves, as the concentration required to give 50% inhibition of specific binding of [³H]-E₂ to the ER, (calculated on the basis of total receptor peak as 100%).
**TABLE 2**

Table 2. Concentrations of phytoestrogens needed to displace 50% [³H]-E₂ bound to type-II EBS in MCF-7 and MDA-MB-468 cells

<table>
<thead>
<tr>
<th>Phytoestrogen</th>
<th>IC₅₀ concentration, nM</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MCF-7</td>
<td>MDA-MB-468</td>
</tr>
<tr>
<td>Genistein</td>
<td>2100</td>
<td>2200</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>590</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td></td>
</tr>
<tr>
<td>Biochanin A</td>
<td>&gt;10000</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td></td>
</tr>
</tbody>
</table>

IC₅₀ values for the compounds were determined from the competition binding curves (as described in methods for type-II EBS) and was calculated as the concentration required to give 50% inhibition of specific binding of [³H]- E₂ to the type-II EBS.
Table 3. IC\textsubscript{50} values for phytoestrogens needed for cell growth inhibition

<table>
<thead>
<tr>
<th>PHYTOESTROGEN</th>
<th>CELL LINE</th>
<th>MCF-7</th>
<th>MDA-MB-468</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC\textsubscript{50}, \mu M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GENISTEIN</td>
<td>37 ± 3.8</td>
<td>8.8 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>QUERCETIN</td>
<td>17.3 ± 2.7</td>
<td>18.1 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>BIOCHANIN A</td>
<td>40 ± 4.1</td>
<td>44 ± 10.1</td>
<td></td>
</tr>
<tr>
<td>KAEMPFEROL</td>
<td>50 ± 3.9</td>
<td>47 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>DAIDZEIN</td>
<td>&gt;100</td>
<td>&gt;100</td>
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</tr>
</tbody>
</table>

Effective concentration of phytoestrogens needed to inhibit the growth of breast cancer cells by 50\% was calculated from growth curves obtained from thymidine incorporation assay, after 72 h of treatment. The values are the average of 2 separate experiments conducted in triplicate.
Table 4. Effect of phytoestrogens on cell cycle distribution in MDA-MB-468 cells after 24 h.

<table>
<thead>
<tr>
<th>PHYTOESTROGEN, 100 μM</th>
<th>% CELLS AT 24 H:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0/G1</td>
</tr>
<tr>
<td>CONTROL</td>
<td>70 ± 6.8</td>
</tr>
<tr>
<td>GENISTEIN</td>
<td>27 ± 4.3</td>
</tr>
<tr>
<td>QUERCETIN</td>
<td>35 ± 5.1</td>
</tr>
<tr>
<td>KAEMPFEROL</td>
<td>50 ± 4.2</td>
</tr>
<tr>
<td>BIOCHANIN A</td>
<td>70 ± 6.1</td>
</tr>
<tr>
<td>DAIDZEIN</td>
<td>60 ± 6.6</td>
</tr>
</tbody>
</table>

Cell cycle distribution was determined using Flow cytometry in MDA-MB-468 control and cells treated with 100 μM phytoestrogens at 24 h. Percentage of cells in each phase of the cell cycle was determined using cytological software.
Table 5. Phytoestrogens and nocodazole treatment on cell cycle distribution in MCF-7 cells

<table>
<thead>
<tr>
<th>% CELLS AT 24 H AFTER TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
</tr>
<tr>
<td>GENISTEIN</td>
</tr>
<tr>
<td>CONTROL</td>
</tr>
<tr>
<td>10 µM</td>
</tr>
<tr>
<td>25 µM</td>
</tr>
<tr>
<td>50 µM</td>
</tr>
<tr>
<td>100 µM</td>
</tr>
<tr>
<td>QUERCETIN</td>
</tr>
<tr>
<td>CONTROL</td>
</tr>
<tr>
<td>10 µM</td>
</tr>
<tr>
<td>25 µM</td>
</tr>
<tr>
<td>50 µM</td>
</tr>
<tr>
<td>100 µM</td>
</tr>
<tr>
<td>NOCODAZOLE</td>
</tr>
<tr>
<td>CONTROL</td>
</tr>
<tr>
<td>0.006 µM</td>
</tr>
<tr>
<td>0.06 µM</td>
</tr>
<tr>
<td>0.6 µM</td>
</tr>
<tr>
<td>6 µM</td>
</tr>
</tbody>
</table>

Cell cycle distribution was determined in MCF-7 cells after treatment with genistein, quercetin and nocodazole for 24 h using Flow cytometric analysis.
FIG. 1. CHEMICAL STRUCTURES OF ESTRADIOL AND PHYTOESTROGENS
FIG. 2. Inhibition of $[^3\text{H}]$-E$_2$ binding to ER by genistein. Cytosol from MCF-7 cells was incubated with 5 nM $[^3\text{H}]$-E$_2$ alone or together with (A) 200-fold excess E$_2$ or (B) increasing concentrations of genistein at 4 °C for 3 h. Free hormones were removed with charcoal and hormone bound receptor was sedimented through a 10-30% linear sucrose gradient. Fractions were collected and counted. $[^{14}\text{C}]$-labeled bovine serum albumin (BSA, 4.5S) and $[^{14}\text{C}]$globulin ($\gamma$G, 7S) were included as internal markers.
FIG. 3. Inhibition of $[^3H]$-E$_2$ binding to human recombinant ER. ER was incubated with radiolabeled E$_2$ alone or with (A) 200-fold excess unlabeled E$_2$ or (B) increasing concentrations of genistein and treated as described in Fig. 2. Arrows indicate sedimentation of $[^{14}C]$-labeled bovine serum albumin (BSA) and $[^{14}C]$globulin (γG).
FIG 4. Competition for type-II EBS by genistein and quercetin in (A) MCF-7 and (B) MDA-MB-468 cells. Cells were incubated with increasing concentrations of phytoestrogens with or without 40 nM or 30 nM [3H]-E2, respectively for 3 h. Type-II EBS were estimated as described in Methods. Non-specific binding was determined using 200-fold excess DES. Results are expressed as per cent [3H]-E2 bound in the absence (100%) or presence of the phytoestrogen.
FIG. 5. Circular dichroism spectra of human recombinant ER in the presence of increasing concentrations of (A) E$_2$ and (B) genistein at 25 °C. The ER was incubated in the storage buffer overnight at room temperature. The spectra were corrected for the contribution from the buffer.
FIG. 6. Breast cancer cell growth was determined based on the amount of DNA synthesized, by thymidine incorporation assay. Cells were treated with genistein for 24, 48 and 72 h respectively in triplicates. One hour before the end of the treatment, cells were pulsed with 1 μCi/ml radioactive thymidine. Radioactivity incorporated into the cellular DNA was measured by scintillation counting. Points are average of two separate experiments conducted in triplicate. Bars are SD. * Significantly different from control, p<0.05.
FIG 7. Flow cytometric analysis showing cell cycle distribution (%) of MDA-MB-468 cells after treatment with 0, 10, 25, 50 and 100 µM concentrations of genistein for 24, 48 and 72 h respectively. Percentage of DNA in G0/G1, S and G2/M phases are depicted.
FIG 8. Western blots showing cyclin B1 levels in MCF-7 cells dosed with 0, 10, 25, 50, 100 µM genistein (Panel A) and 0, 0.006, 0.06, 0.6 and 6 µM nocodazole (Panel B) for 24 h.
FIG 9. Western blots showing cyclin B1 levels after treatment with genistein at 0, 10, 25, 50, 100 μM concentrations (lanes 1 through 5, respectively) or quercetin at 0, 10, 25, 50 and 100 μM concentrations (lanes 6-10, respectively), in MDA-MB-468 cells. 0 h represents control cells at time zero.
FIG 10. MCF-7 cells were treated with genistein for 4 h (Panel A) and for 8, 12 and 24 h (Panel B). The cells were then harvested and ODC activity determined as described in Methods. Results are an average of triplicate experiment. Bars represent SD.
EFFECT OF GENISTEIN TREATMENT ON INTRACELLULAR POLYAMINE LEVELS IN MCF-7 CELLS

A. PUTRESCINE

B. SPERMIDINE

C. SPERMINE

FIG 11. MCF-7 cells were treated with 0, 10, 25, 50 and 100 μM genistein for 24, 48 and 72 h. The cells were harvested after the appropriate time and Putrescine (Panel A), Spermidine (Panel B) and Spermine (Panel C) levels were determined as described in Methods. The results are an average of 2 separate experiments conducted in triplicate. Bars represent SD.
APPENDIX 1

Acronym and symbol definitions in alphabetical order

CD             Circular dichroism
DCC            Dextran coated charcoal
DES            Diethyl stilbestrol
DMEM           Dulbecco’s minimum essential medium
DMSO           Dimethyl sulfoxide
EBS            Estrogen binding sites
ER             Estrogen receptor
E2             Estradiol
FBS            Fetal bovine serum
HPLC           High performance liquid chromatography
MEM            Minimum essential medium
ODC            Ornithine decarboxylase
[3H]-E2        Tritiated estradiol
rER            Recombinant estrogen receptor
**PREVENTION 8**


Oiltarp: a synthetic D-thiolethionoate and genistein an isoflavone and component of soy products have both been shown to exhibit potential chemopreventive effects in vitro and in vivo. In this study we have examined the effect of these suspected anticarcinogens in vivo on DNA adducts induced by the experimental carcinogen, dibenz[a,j]pyrene (DBP). Female SD rats were provided diet containing (oiltarp 500 µg/kg diet, genistein 100 µg/kg diet) or control diet for 7 days prior to a single intraperitoneal injection of DBP (2.2 mg/kg, b.w.). The animals remained on this diet for 5 days post-DBP dosing and were sacrificed at 4 h post-dosing with DMSO in the formation of 1 µM DNA adducts in the mammary gland and lung while several additional adduct spots were detected in the liver. Adducts were the highest in the mammary gland with levels of 121 ± 48 µM adducts/liver while in the lung and liver adducts were 5-10-fold lower with levels of 44 ± 13 and 45 ± 16 µM adducts/liver, respectively. Genistein, a natural isoflavone of soybeans, inhibits growth and induces terminal differentiation of mammary tumor cells. We also show that sensitivity of the cells to genistein’s action depended on cellular p53. Based on these observations and the report that genistein treatment causes a double-strand breaks in DNA, we hypothesized that p53 plays a role in genistein’s action. In the present study, we sought to determine whether p53, p53-mediated, and MDM2 induction occurs in the mammary gland response pathways leading to cell cycle arrest following genistein treatment. The experimental protocol was similar to that described above, except that 10 µM p53 was used in our investigation. We used p53 in the mouse mammary gland. The results showed that genistein-induced p53, p52, and MDM2 in a dose-dependent manner, induction of p53 serum level increases. The role of p53 in the regulation of p58 is of particular interest. The results were obtained from American Cancer Society National Grant.

**Induction of p53, p21, and MDM2 are: associated with G2/M arrest in metastatic melanoma cells treated in vitro.** Reuth, S., Bodei, E., Pezzuto, J., Pisha, E., Green, A. Dept. of Surgical Oncology, University of Illinois at Chicago, IL 60612 USA.

Genistein, a major isoflavone of soybeans, inhibits growth and induces terminal differentiation of metastatic melanoma tumor cells. The present study shows that genistein significantly inhibits growth and induces differentiation in metastatic melanoma cells. We also show that sensitivity of the cells to genistein’s action depended on cellular p53. Based on these observations and the report that genistein treatment causes a double-strand breaks in DNA, we hypothesized that p53 plays a role in genistein’s action. In the present study, we sought to determine whether p53, p53-mediated, and MDM2 induction occurs in the mammary gland response pathways leading to cell cycle arrest following genistein treatment. The experimental protocol was similar to that described above, except that 10 µM p53 was used in our investigation. We used p53 in the mouse mammary gland. The results showed that genistein-induced p53, p21, and MDM2 in a dose-dependent manner, induction of p53 serum level increases. The role of p53 in the regulation of p58 is of particular interest. The results were obtained from American Cancer Society National Grant.

The natural tyrosine kinase inhibitor genistein inhibits growth and induction of sarcoma cell lines in vitro. Reuth, S., Green, A., Das Gupta, T.K., Borstein, A. Dept. of Surgical Oncology, University of Illinois at Chicago, IL 60612 USA.

Genistein (4',5,7-trihydroxyisoflavone) demonstrated strong growth inhibitory effects against many solid tumors both in vitro and in vivo. Our current studies show that it inhibits growth in metastatic melanoma cell lines. We also showed that the chemosensitivity of the cells depended on the status of cellular p53. In the present study, we investigated genistein’s effect on human sarcoma cell lines in culture. The fibrosarcoma cell line HT1080 (positive), the osteosarcoma cell line SAOS-2 (negative) and the Ewing sarcoma cell line UISO-ES3 (positive) were used in our study. Those were treated with different concentrations of genistein for different time periods. The results showed that genistein significantly inhibited cell adhesion in a dose- and time-dependent manner. Treatment with 100 µM of genistein resulted in complete inhibition of cell adhesion and growth in 24 h. To 200 µM concentrations resulted in loss of adhesion of 50% of the cells. The results were obtained from American Cancer Society National Grant.


Breast cancer is one of the leading causes of cancer death among women. Epidemiologic studies have suggested a relationship between diets high in soy and a low incidence of breast cancer. It has been demonstrated that consumption of genistein in soy isoflavonoids found in soy first in chemoprotection against DMBA-induced mammary carcinogenesis in rat model. Since most of the soy consumption is through diet, it is necessary to know if soy diets have similar effects as injections of purified isoflavones. In the present study, female Sprague-Dawley were maintained on AIN-93 diet which sources of the protein were casein and corn. Protein four weeks prior to breeding and continued throughout the study. At day 50 of age, all female pups from both diets received via gavage 80 mg/kg dimethylnitrosamine (DMBA). All rats were palpated twice weekly for mammary tumors and they were killed when 100% rats on the casein diet developed tumors. Rats on the soy diet took a longer period of time for mammary tumors to develop compared to rats on the casein diet (2 weeks). At the end of the experiment rats in the diet had an 84% induction of tumors compared to 100% of rats on the casein diet. The number of tumors per rat on soy diets were less (6.74 vs. 3.81) rats on casein diet. These results suggest that dietary soy consumption can protect against the development of DMBA-induced mammary tumors. Supported by USDA-2-521-510-001-025.


Genistein (4',5,7-trihydroxyisoflavone) is a constituent of soy foods, the consumption of which is believed to contribute to the low incidence of breast cancer in oriental women. In order to understand the mechanism of action of genistein, we studied the effects of genistein on long-term cell growth, cell cycle phase kinetics, and cyclin B1 accumulation using MCF-7 breast cancer cells. Genistein inhibited cell growth in a dose-dependent manner, with an IC50 value of 50 µM as determined by total DNA content per a period of 8 days. At 100 µM concentration, genistein inhibited cell proliferation, and DNA synthetic analysis of cells treated with 100 µM genistein for 24 h showed a 2- to 3-fold increase in the level of cyclin B1 protein in cells treated with genistein compared to the control time point. These data suggest a possible mechanism for the action of genistein in breast cancer cells: due to an accumulation of cyclin B1 in breast cancer cells: Future studies will include an understanding of the nature of genistein interation(s) with cyclin/cdk complexes.

This work was supported by a pre-doctoral Fellowship from the U.S. Breast Cancer Research Program (DAMD17-97-1-7035).


The low prostate cancer rates in Asian countries may be attributable to high dietary intakes of soy. The major soy isoflavone genistein inhibits growth of human prostate cancer cell lines LNCaP, PC3, and DU145. We examined the mechanism of these growth inhibiting effects of genistein at non-cytotoxic (dye-exclusion) concentrations of 1.65-8.53 µM; genistein serum levels in Japanese consuming traditional diets are approx. 6 µM. Tritiated thymidine uptake was reduced dose-related at all concentrations in all 3 lines. Genistein induced apoptosis in LNCaP cells (TUNEL assay). This was confirmed by flow cytometry, with sub-G1 cells being hypodiploid after 72 h exposure to a high dose of genistein. In contrast, genistein caused senescence in PC3 and DU145 cells. The PC3 and DU145 lines have inactivating p53 mutations. PC3 and DU145 express wild-type p53 which, in response to cellular stresses, transcriptionally activates the promt G1 arrest and apoptosis. Genistein, similar to etoposide as a positive control, increased apoptosis in LNCaP cells, but not in DU145 cells of the anticancer p53 target gene BTG2 in a dose-related fashion was reduced after 72 h exposure. PC3 and DU145 cells did not express BTG2. These data suggest involvement of p53 in growth inhibition of LNCaP but not PC3 or DU145 cells by genistein. (Supported by CA72200 and CaP CURE).

**Potential chemopreventive properties of glutathione conjugate of benzyliselenoacétate against colon carcinogenesis in rats.** Reedy, B.S., Kawamoto, T., Raja, J., and B-Bayoumy, K. American Health Foundation, Valhalla, NY 10595.

The major goal of this study was to develop organoselenium compounds with maximal chemopreventive efficacy yet lowest possible toxicity. The chemopreventive efficacy of organoselenium compounds depend on the form in which they are administered, supported, taking into account their bioavailability. The preliminary results indicate that glutathione conjugates of chemopreventive organoselenium compounds, benzyliselenoacétate (BSC) and 1-anethole selenoacétate [(methylthio)iselenoacétate (p-XSC)-S]-are putative metabolites that may be responsible for their biological effects. The present study was designed to evaluate the potential chemopreventive activity of glutathione conjugates of BSC and BuS-Se (BSC-Se-GA) against colon aberrant crypt foci (ACF), which are prevalent lesions in the colon. BSC and BuS-Se were synthesized in our laboratory. Male F344 rats were fed the control and experimental diets for 8 weeks. All rats were fed (1 g of BSC (4.1 ppm Sc) and 10 and 20 ppm BuS-Se (1.8 and 3.6 ppm Se) starting on day one prior to the first of two weekly s.c. injections of azoxymethane (15 mg/kg body wt.) and until the animals were killed 8 weeks later for ACF evaluation. Administration of BSC and BuS-Se significantly suppressed the colonic ACF. Additionally, the degree of inhibition of ACF is more pronounced with BuS-Se in a dose-dependent manner.
The 1998 Annual Retreat on Cancer Research in New Jersey

May 29, 1998

GALLO AWARD

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The Cancer Institute of New Jersey

For Outstanding Cancer Research
Srivani Balabhadrapatruni

Research Titled

Genistein Treatment Causes G2/M Cell Cycle Arrest and Cyclin B1 Accumulation in Breast Cancer Cells: A Possible Mechanism for the Preventive Action of Genistein

T. S. Ravikumar, MD
Associate Director for Education and Training
The Cancer Institute of New Jersey
Co-Chair, The 1997 Annual Retreat on Cancer Research in New Jersey

William N. Hait, MD, Ph.D.
Director, The Cancer Institute of New Jersey
Professor of Medicine & Pharmacology
UMDNJ - Robert Wood Johnson Medical School