Genistein Potentiates the Radiation Effect on Prostate Carcinoma Cells

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ABSTRACT

We have shown previously that genistein, the major isoflavone in soybean, inhibited the growth of human prostate cancer cells in vitro by affecting the cell cycle and inducing apoptosis. To augment the effect of radiation for prostate carcinoma, we have now tested the combination of genistein with photon and neutron radiation on prostate carcinoma cells in vitro. The effects of photon or neutron radiation alone or genistein alone or both combined were evaluated on DNA synthesis, cell growth, and cell ability to form colonies. We found that neutrons were more effective than photons for the killing of prostate carcinoma cells in vitro, resulting in a relative biological effectiveness of 2.6 when compared with photons. Genistein at 15 μM caused a significant inhibition in DNA synthesis, cell growth, and colony formation in the range of 40–60% and potentiated the effect of low doses of 200–300 cGy photon or 100–150 cGy neutron radiation. The effect of the combined treatment was more pronounced than with genistein or radiation alone. Our data indicate that genistein combined with radiation inhibits DNA synthesis, resulting in inhibition of cell division and growth. Genistein can augment the effect of neutrons at doses ~2-fold lower than photon doses required to observe the same efficacy. These studies suggest a potential of combining genistein with radiation for the treatment of localized prostate carcinoma.

INTRODUCTION

Carcinoma of the prostate is the most common malignant tumor in men, with >180,400 newly diagnosed cases annually, resulting in >31,000 deaths each year (1). Localized prostate carcinoma is sensitive to conventional radiotherapy using megavoltage photons (X-rays); however, residual disease often causes clinical relapse (2). To increase the efficacy of radiation therapy, the use of neutrons was explored. Neutrons are heavy particles produced when a charged particle, such as deuterium, is accelerated to high energy and then made to impinge on a target such as beryllium (3). The interaction of neutrons with nuclei of atoms of soft tissues sets in motion heavy secondary particles, which is less repairable than that induced by photons. Neutrons are both more effective in killing hypoxic tumor cells and less dependent on the cell cycle than photons (3). These differences between neutrons and photons result in a greater RBE for neutrons relative to photons and form the radiobiological basis for selecting neutrons for therapy of slow-growing tumors, such as adenocarcinoma of the prostate. In the treatment of locally advanced adenocarcinoma of the prostate, neutrons have been shown to be superior to photons in two randomized multi-institutional Phase III clinical trials (4, 5).

At Wayne State University, we have improved the conditions for three-dimensional conformal neutron irradiation using a superconducting cyclotron fully rotational around the patient to produce an isocentric beam operated with a tungsten multirod collimator used to produce irregularly shaped fields (6, 7). Field apertures were designed to conform to the size and shape of the three-dimensional reconstructed tumor volume. After a series of Phase II/III trials for localized and locally advanced prostate carcinoma using these techniques, we have established a regimen of neutrons combined with photons that results in a significant decrease in tumor recurrence and lower toxicity than that observed in the previous trials (8, 9). At 3 years, no evidence of recurrence of disease was found in 91% of patients with stage T1 disease, in 86% of stage T2 disease, and 61% of stage T3/T4 disease (9). Although the local control of stage T3/T4 (61%) was improved over photon radiation alone (35–40%), these findings showed that this treatment alone was insufficient to prevent progression of disease in a large proportion of patients. To improve the local control of advanced disease, radiation should be combined with additional antitumor agents. In this study, our goal was to test whether genistein could augment the efficacy of radiation for the treatment of prostate cancer. We have shown previously that genistein inhibits the growth of human prostate cancer cells in vitro by inducing apoptosis (10). Genistein is an isoflavone, a major metabolite of soy produced by the intestinal

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The abbreviations used are: RBE, relative biological effectiveness; CM, culture medium; NF-κB, nuclear factor-κB.
bacteria, that is believed to be one of the anticancer agents found in soybeans (11, 12).

The interest in genistein stems from observations that increased soy consumption in Asian diets, resulting in increased serum isoflavone levels, has been associated with a decreased risk for prostate adenocarcinoma. Epidemiology studies have shown that Japanese and Chinese men have the lowest incidence of prostate carcinoma in the world (13). Genistein has an heterocyclic diphenoic structure similar to estrogen (14) and has demonstrated antitumor and antiangiogenic activities (15, 16). Genistein was found to inhibit tyrosine protein kinases (except p40 protein-tyrosine kinase; Ref. 17), topoisomerases I and II (18), protein histidine kinase, and 5a-reductase (19). Genistein has been shown to inhibit cell growth of tumor cell lines from various malignancies including breast, lung, melanoma, prostate, head and neck squamous cell carcinoma, leukemia, and lymphoma (10, 20–26). We have shown previously that genistein inhibited the cell growth of androgen-dependent (LNCaP) and androgen-independent (PC-3) human prostate carcinoma cell lines (10). We found that genistein induced a G2-M cell cycle arrest leading to cell growth inhibition (10). Cell growth inhibition was observed with concomitant down-regulation of cyclin B, up-regulation of the p21WAF1 growth-inhibitory protein, and induction of apoptosis (10). This effect of genistein on disruption of the cell cycle, resulting in the inhibition of cell growth of prostate cancer cells, suggests that genistein can be used to augment the cell killing induced by radiation. The goal of our study was to investigate in vitro the effect of photon and neutron radiation on PC-3 cells and test whether genistein pretreatment of PC-3 cells could augment the cell growth inhibition induced by either form of radiation. This study shows that genistein potentiates the effect of low doses of photon or neutron radiation, resulting in a significant increase in cell killing of human prostate cancer cells in vitro.

MATERIALS AND METHODS

Tumor Cell Line. The human prostate carcinoma PC-3 tumor cell line was purchased from American Type Culture Collection (Rockville, MD). PC-3 cells were cultured in CM consisting of F-12 K nutrient mixture supplemented with 7% heat-inactivated fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 2 mM glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate (Sigma Chemical Co., St. Louis, MO), 10 mM HEPES buffer, 100 units/ml penicillin/streptomycin, 0.5 μg/ml fungizone, and 50 μg/ml gentamicin.

[3H]Thymidine Uptake Assay. Cells were seeded in six replicates in 96-well flat-bottomed microplates at 3000 cells/well/0.1 ml in CM. After 24 h, genistein was added in 0.05 ml of CM, resulting in a final concentration of 1–90 μM. After 24 h exposure to genistein, cells in the microplates were irradiated with various doses of photons or neutrons. In all wells, the volume was adjusted to 0.2 ml with CM. After 3 days incubation at 37°C in a 5% CO2 incubator, cells were labeled with 1 μCi of [3H]thymidine (Amersham) for 18 h (27, 28). Plates were harvested onto a glass fiber filter with a micromate 196 harvester (Packard Instrument, Meriden, CT). Filters were counted in the β direct Matrix 96 counter (Packard Instrument, Meriden, CT; Refs. 27 and 28). The percentage of inhibition was calculated as follows:

\[
\% \text{ inhibition} = \frac{\text{Untreated cpm} - \text{treated cpm}}{\text{Untreated cpm}} \times 100
\]

Tumor Cell Growth and Colony Assays. Cells were plated in T25 flasks at 0.5 × 10^6 cells/flask in CM. Three days later, cells were treated with genistein at a final concentration of 1–90 μM. After 24 h exposure to genistein, cells were removed by trypsinization using trypsin-EDTA (Life Technologies, Inc.), counted, and transferred to 15-ml conical tubes at about 2 × 10^6 cells/5 ml in CM for irradiation with various doses of photons or neutrons. After radiation, cells were either plated for a cell count or for colony assay (29). For a cell count, cells were plated in duplicates in T25 flasks at 0.5 × 10^6 cells/flask and supplemented with genistein at a final concentration of 5–30 μM. On day 3 of incubation at 37°C in a 5% CO2 incubator, cells were removed by trypsinization and counted using eosin exclusion dye. The mean cell number of duplicates was used for data analysis. To set up the colony assay after radiation, cells were plated in triplicates in six-well plates at 250 cells/well and supplemented with genistein at a final concentration of 5–30 μM. After 10 days incubation at 37°C in a 5% CO2 incubator, colonies were fixed in 10% buffered formalin, stained with eosin and hematoxylin, and counted (29). The percentage of inhibition of the cell number or colonies was calculated as follows:

\[
\% \text{ inhibition} = \frac{\text{Untreated} - \text{treated}}{\text{Untreated}} \times 100
\]

Radiation. Cells, in 96-well microplates or 15-ml tubes, were either irradiated with neutrons or photons. For neutron irradiation, the beam was produced by bombarding a thick beryllium target with 48.5 MeV deuterons [d(48.5)+Be] from a superconducting cyclotron (6, 7). The microplates were irradiated from above with a vertical beam. They were covered with 1 cm of A150 tissue-equivalent plastic (Exradin, Inc., Lisle, IL) to provide adequate build-up and to flatten the 25 × 25-cm field. The distance between the target and the surface of the plastic was 170 cm. The total dose rate with this arrangement was ~51 cGy min^-1 with 12.5 μA of deuteron beam. Tubes were irradiated from above with a vertical beam. They were covered with 1 cm of A150 tissue-equivalent plastic (Exradin, Inc., Lisle, IL) to provide adequate build-up and to flatten the 25 × 25-cm field. The distance between the target and the surface of the plastic was 170 cm. The total dose rate with this arrangement was ~51 cGy min^-1 with 12.5 μA of deuteron beam. For both arrangements, there was an approximately 3–5% γ-ray dose component.

Photon irradiation was performed with a 60Co unit (AECL Theratron 780). Arrangements similar to those used for neutron irradiation were used for 60Co irradiation. Microplates were irradiated from above with a vertical beam. 2.5 mm of polystyrene build-up material was placed on top of the flasks, and the surface of the build-up material was at a distance of 76 cm from the source. The dose rate was ~32 cGy min^-1. For tube irradiation, a Lucite block similar to that used for neutron irradia-
tion was used with a horizontal beam. The surface of the block was positioned at 46 cm from the source, the tubes were at a depth of 2.6 cm, and the dose rate was ~92 cGy min$^{-1}$.

**Statistical Analysis.** Statistical analysis of the data was carried out using the BMDP Statistical Software version 7.0. For the thymidine assay, outcome values (cpm) were evaluated using a two-way ANOVA. Multiple comparisons were conducted using the Bonferroni procedure. For the colony assay, preliminary data analysis indicated that two-way ANOVA models were inappropriate because of substantial statistical interaction between radiation and genistein. An additional problem was heterogeneity of variances. Consequently, we used one-way ANOVA to compare the effects of different radiation and genistein doses. When Bartlett’s test indicated significant heterogeneity of variance, the analyses were repeated on square root transformed data. In the case where there was still significant heterogeneity of variances, two-sample $t$ tests were performed using Satterthwaite’s method for unequal variances. Bonferroni’s procedure was used to correct for multiple comparisons.

**RESULTS**

**PC-3 Cell Growth Inhibition Induced by Photon Radiation and Neutron Radiation.** To test for the radiosensitivity of the human PC-3 prostate carcinoma cell line to photons and neutrons, cells were irradiated with a wide dose range of either photons or neutrons. Irradiated cells were assayed in a colony assay to compare the long-term effects of photon and neutron radiation on cell division and formation of colonies. Radiation of PC-3 cells induced a dose-dependent inhibition of colony formation observed with both types of radiation (Fig. 1). However, neutrons used at lower doses than photons were more effective than photons. A significant inhibition of ~50% was obtained at 100 cGy neutrons and at 200–300 cGy photons. The RBE calculated as the ratio of photon dose to neutron dose causing 50% inhibition was found to be ~2.6 for PC-3 cells.

**DNA Synthesis Inhibition by Genistein and Radiation.** To investigate the effect of genistein combined with radiation on DNA synthesis of PC-3 cells, cells in microplates were first treated with genistein for 24 h and then irradiated with photon or neutron radiation. Photon and neutron doses were selected based on the dose titration experiments shown in Fig. 1. After radiation, the cells in microplates were further incubated for 3 days, and DNA synthesis of untreated and treated PC-3 cells was monitored by [$^3$H]thymidine incorporation. In this assay, cells were continuously exposed to genistein, even after radiation for the duration of the experiment (4 days). Data from a representative experiment are presented in Fig. 2. A dose-dependent decrease in [$^3$H]thymidine counts was measured with increasing concentrations of genistein from 5 to 60 $\mu$M, indicative of inhibition of DNA synthesis (Fig. 2A). The extent of DNA inhibition induced by genistein was further enhanced by increasing doses of photons (Fig. 2A). The percentage of DNA inhibition relative to control untreated cells was calculated to compare between the combinations of various doses of genistein and radiation (Fig. 2B). Photon irradiation caused a gradual and significant increase in DNA inhibition from 28 to 72% at doses of 100–500 cGy compared with untreated control cells ($P < 0.01$). At lower concentrations of genistein of 1 and 5 $\mu$M, the effect of genistein alone was minimal ($P > 0.01$), and no significant increase in DNA inhibition was observed with radiation above that induced by radiation alone (Fig. 2B). At concentrations of 30 $\mu$M genistein and higher, the effect of genistein alone was >90% DNA inhibition ($P < 0.01$), and only a slight further increase to 96% was seen by adding radiation. However, genistein at a concentration of 15 $\mu$M caused a significant 46% inhibition of DNA synthesis compared with untreated cells ($P < 0.01$) that was further augmented by radiation at each dose of radiation tested in a radiation dose-dependent manner (Fig. 2B). The DNA inhibition induced by 15 $\mu$M genistein combined with photon radiation at doses of 100–500 cGy (67–91%) was significantly greater than that induced by genistein alone (46%; $P < 0.01$) and radiation alone (28–72% at 100–500 cGy; $P < 0.01$). This effect was marked at intermediate doses of radiation of 200 and 300 cGy inducing 78 and 83% DNA inhibition, respectively, compared with 37 and 53% inhibition induced by 200 and 300 cGy photons alone ($P < 0.01$).

The combination of genistein with neutron radiation on PC-3 cells followed a pattern of DNA synthesis inhibition comparable with that observed with genistein combined with photons. Data from a representative experiment are presented in Fig. 3. In this experiment, the dose range of genistein was selected to include concentrations between 15 and 35 $\mu$M based on data shown in Fig. 2. The dose-dependent decrease in [$^3$H]thymidine counts induced by genistein was comparable with that seen in the independent experiment shown in Fig. 2 showing the reproducibility of the genistein effect on DNA synthesis. This effect was also consistently increased by additional exposure of the cells to increasing doses of neutrons (Fig. 3A). Genistein at concentrations of 15 and 25 $\mu$M, respectively, induced 47 and 78% DNA inhibition compared with untreated cells ($P < 0.01$; Fig. 3B). This DNA inhibition was significantly increased to 66–93% inhibition by combination with low doses...
of 50–200 cGy neutron radiation compared with genistein alone (P < 0.01) and to the 47–68% inhibition induced by 50–200 cGy neutrons alone (P < 0.01; Fig. 3B). These data indicate that genistein can also augment the effect of neutrons at doses ~2-fold lower than photon doses required to observe the same efficacy. This experiment also shows that concentrations of genistein >30 μM have an optimal effect alone, as seen previously in Fig. 2B.

Both for photons + genistein and neutrons + genistein, the effect on DNA synthesis inhibition does not follow an additive pattern when compared with each treatment separately but rather follows a pattern indicating potentiation of radiation effect by pretreatment with genistein.

**Cell Growth Inhibition by Genistein and Radiation.** To study the effect of DNA synthesis inhibition induced by genistein and radiation on cell growth, cells were pretreated with genistein for 24 h and then irradiated and incubated in the presence of genistein. The short-term effects of the combined treatment on cell growth were assessed by counting viable cells 3 days after radiation, whereas the long-term effects on cell division were assessed in a colony assay on day 10 after radiation. On day 3, the cell count showed the same trend observed in DNA synthesis with the combined treatment of 15 μM genistein with 50–200 cGy neutrons.
genistein and 300 cGy photons causing 69% inhibition in cell growth compared with 50% with each treatment alone (Table 1). This extent in cell growth inhibition was lower than the 83% observed in DNA inhibition using the same doses of genistein and radiation and indicates that cell death is a slower event than DNA synthesis inhibition. Cell growth inhibition was not enhanced further by radiation combined with 30 \( \mu \text{M} \) genistein. With low doses of neutrons combined with genistein, the effect on cell growth was comparable with that observed with genistein and photons; however, an increased inhibition in cell growth was also observed at 5 \( \mu \text{M} \) genistein combined with 100 or 150 cGy neutrons (Table 1). Although variations in cell growth inhibition by genistein is observed in the two representative experiments presented in Table 1, the trend of increased cell growth inhibition by genistein combined with radiation remains.

The long-term effects of the combined treatment on cell division as measured by colony formation on day 10 posttreatment were more pronounced than those observed on day 3 (Fig. 4). Genistein alone at 30 \( \mu \text{M} \) caused a complete inhibition of 97–100% in colony formation (data not shown). In the photon/genistein experiment (Fig. 4A), a minimal effect was observed at 5 \( \mu \text{M} \) (\( P = 1.00 \)), whereas a significant inhibition of 67% in colony formation was seen at 15 \( \mu \text{M} \) genistein (\( P < 0.001 \)). Photons at 200 and 300 cGy caused a significant inhibition of 38 and 55%, respectively, in colony formation (\( P < 0.001 \)). A slight increase in colony inhibition was obtained by combination of 5 \( \mu \text{M} \) genistein and 200 or 300 cGy photons, which was not significant compared with radiation alone (\( P > 0.2 \)). However, when these radiation doses were combined with 15 \( \mu \text{M} \) genistein, a significant increase in colony inhibition was observed up to 75–84% with 200 and 300 cGy photons that was significantly greater than with radiation alone (\( P < 0.002 \)). This effect on cell division corroborates the effect observed in DNA synthesis.

In the neutron/genistein experiment (Fig. 4B), the effect of 50 cGy neutrons alone on colony formation was not significant (\( P = 1.00 \)), whereas a significant inhibition in colony formation was observed at 100 and 150 cGy neutrons (\( P < 0.05 \)). In this experiment, some inhibition in colony formation (26%) was observed with 5 \( \mu \text{M} \) genistein alone, indicating variations between experiments when compared with the photon/genistein experiment (Fig. 4A). The combination of 5 \( \mu \text{M} \) genistein with radiation at doses of 50 and 100 cGy neutrons showed a further decrease in the number of colonies that was significant to genistein alone (\( P < 0.001 \)) and to radiation alone (\( P < 0.05 \)). The effect of 15 \( \mu \text{M} \) genistein alone was more reproducible and more significant (59% inhibition; \( P = 0.001 \)). At neutron doses of 100 and 150 cGy combined with 15 \( \mu \text{M} \) genistein, a significant increase in colony inhibition to 86 and 95% was observed compared with 60% with genistein alone (\( P < 0.001 \)) and compared with 49 and 77% with 100 and 150 cGy neutrons alone, respectively (\( P < 0.001 \); Fig. 4B). This effect was greater than that observed in DNA division. The effect of 15 \( \mu \text{M} \) genistein and 100 cGy neutrons was comparable with 15 \( \mu \text{M} \) genistein combined with 300 cGy photons, resulting in >80% inhibition in colony formation. These data show that much lower doses of neutrons than photons are sufficient for a genistein potentiation effect. The effect of genistein combined with neutrons seems more pronounced on cell division than that obtained with genistein and photons because the combination of genistein and neutrons was significantly better than genistein alone or neutrons alone.

Microscopic observation of the colonies in the combined treatment showed that cells failed to form colonies, and remain-

**Table 1** Effect of genistein and radiation on cell growth

<table>
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<th>Experiment</th>
<th>Genistein (( \mu \text{M} ))</th>
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<td>Neutron radiation (cGy)</td>
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<td></td>
<td>150</td>
<td>32</td>
<td>54</td>
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**Fig. 4** Colony formation of PC-3 cells treated with genistein and radiation. PC-3 cells were treated for 24 h with genistein and then irradiated with photons or neutrons in two separate experiments. After radiation, cells were plated at 250 cells/well in a colony assay. The colonies were stained and counted after 10 days incubation as described in “Materials and Methods.” The mean number of colonies calculated on triplicate wells is reported; bars, SE.
form a colony (120). (large arrow) with large cytoplasmic vacuoles. These cells failed to arrowheads empty arrow). Note part of a giant cell curved arrows cytoplasmic vacuolization (small), disintegrating nuclei (120).

Typical pleomorphic morphology of PC-3 cells with large nuclei and ing cells were enlarged, showing intense cytoplasmic vacuolization and disintegrating nuclei compared with the morphology of untreated cells (Fig. 5). These findings are similar to the effect of genistein alone on the cell morphology (10) and to the effect of radiation alone on the cell morphology.4

It should be noted that the cell growth inhibition induced by genistein alone or combined with radiation is dependent on the presence of genistein during the 10 days incubation of the colony assay. In separate experiments in which cells were pretreated with genistein for 24 h and then irradiated and plated in the colony assay for 10 days with no genistein, the effect of genistein was much milder. Thus, continuous exposure before and after radiation is necessary for an optimal effect of the combined treatment.

Optimal Conditions for Genistein and Radiation Interaction. We have demonstrated that the effect of a low dose of 15 μM genistein is sufficient to induce about 50–60% growth inhibition of PC-3 cells. This dose was found to be optimal for combination with low doses of radiation of 100–300 cGy photons or 100–150 cGy neutron radiation and resulted in up to 80% inhibition in DNA synthesis and 84–95% inhibition in cell division. Fig. 6 emphasizes these findings by comparing the inhibitory effect of the combined treatment on DNA synthesis and cell division. These data show a good correlation between both [3H]thymidine and colony assays and indicate that the DNA synthesis inhibition observed after 4 days exposure to genistein with and without radiation is irreversible and does lead to cell division inhibition measured by the colony assay.

DISCUSSION

The efficacy of neutrons combined with photons was demonstrated in clinical trials for localized prostate carcinoma at stages of T1 and T2 (8, 9). A better local control of stage T2/T4 was also obtained with neutron/photon regimen than that observed with photons alone (8, 9). To improve further the outcome of patients with locally advanced disease, we have tested the combination of photon and neutron radiation with the soy isoflavone genistein on prostate carcinoma cells in vitro. The advantage of genistein over other antitumor agents is that genistein at low doses can be safely used in patients. Soy products containing 30–200 mg of isoflavones are widely available in food, nutrition, and vitamin stores. Humans consuming soy have micromolar concentrations of isoflavones in their blood. We have shown previously that genistein inhibits the growth of human prostate cancer cells in vitro by affecting the cell cycle and inducing apoptotic processes (10). The rationale for the combination of genistein and radiation was to expose the cells to two cytotoxic modalities that may act differently in the molecular pathways, leading to cell death and resulting in greater cell killing.

Radiation of PC-3 cells either with neutrons or photons induced a dose-dependent inhibition of colony formation. However, we demonstrated that neutrons were more effective than photons with an RBE of 2.6 for PC-3 cells. We determined a dose range of photons (100–500 cGy) and neutrons (50–200 cGy) causing a significant effect on cell division (30–70% inhibition) but not a complete inhibition that is adequate for a combination with genistein.

We have shown previously that genistein significantly inhibited the growth of PC-3 cells at concentrations >10 μM when cells were counted after 3 days in culture (10). In this study, similar findings were obtained. We found that 15 μM genistein also inhibited PC-3 cell growth, in the range of 30–50%, after continuous exposure for 3 days. In addition, we showed that the decrease in cell growth was attributable to DNA synthesis inhibition (>45%), as measured in a 4-day assay leading to cell division inhibition of 60–70% in the 10-day colony assay. These data confirm our previous findings that genistein causes cell cycle arrest leading to cell killing (10). Therefore, the dose of genistein selected for combination with radiation was 15 μM because it induced a significant and median inhibition in DNA synthesis, cell growth, and colony formation in the range of 40–60%. A lower dose of 5 μM genistein was not as effective, and doses higher than 30 μM were toxic to the tumor cells, as measured in the three assays.

Fig. 5 Morphology of cells from day 10 colonies treated with genistein combined with radiation. Prints from colonies obtained in the experiment described in Fig. 4 are shown. A, colony from untreated cells. Note typical pleomorphic morphology of PC-3 cells with large nuclei and prominent nucleoli (×120). B, colony from PC-3 cells treated with 15 μM genistein and 300 cGy photons showing large cells with intense cytoplasmic vacuolization (curved arrows), disintegrating nuclei (small empty arrow), or apoptotic cells (arrowheads). Note part of a giant cell (large arrow) with large cytoplasmic vacuoles. These cells failed to form a colony (×120).

4 Personal communications.
Pretreatment of PC-3 cells with genistein for 24 h, followed by radiation with photons and continuous exposure to genistein for 3 more days, caused a significant increase in DNA synthesis inhibition that was greater than that induced by genistein or radiation alone. This effect was particularly observed at 15 μM genistein combined with intermediate doses of radiation of 200 and 300 cGy inducing 78 and 83% DNA inhibition, respectively, compared with 37 and 53% inhibition induced by 200 and 300 cGy photons alone and with 46% inhibition caused by genistein alone. This augmentation of DNA inhibition by genistein and photon radiation was also induced by pretreatment with genistein followed by neutron radiation. This DNA inhibition was significantly increased to 66–93% inhibition by combination with low doses of 50–200 cGy neutron radiation compared with genistein alone (47%) and with the inhibition induced by 50–200 cGy neutrons alone (47–68%). These data indicate that genistein can also augment the effect of neutrons at doses ~2-fold lower than photon doses required to observe the same efficacy. Both for photons + genistein and neutrons + genistein, the effect on DNA synthesis inhibition does not follow an additive pattern when compared with each treatment separately but rather follows a pattern indicating potentiation of radiation effect by pretreatment with genistein. Thus, we believe that genistein may function as a radiosensitizer for PC-3 cells.

When cell growth was measured after treatment with genistein and radiation, a further decrease in the cell number was observed compared with genistein alone and radiation alone. These data showed that a trend in cell growth inhibition comparable with that observed in DNA synthesis inhibition was obtained, indicating that inhibition of DNA synthesis may have resulted in decreased cell growth. The long-term effects of the combined treatment on cell division as measured by colony formation on day 10 after treatment were markedly increased compared with those observed on day 3. The increased inhibition of colony formation was particularly pronounced at 15 μM combined with 300 cGy photons or 100–150 cGy neutrons, causing up to 80–95% inhibition. This effect on cell division corroborates the effect observed in DNA synthesis but shows that cell death may be a late event, as observed previously for genistein alone (10) and radiation alone. These data show a good correlation between both [3 H]thymidine and colony assays and indicate that the DNA synthesis inhibition observed after 4 days exposure to genistein and radiation is irreversible and does lead to cell division inhibition measured by the colony assay.

Fig. 6  Optimal inhibition of cell division and growth by genistein and radiation in PC-3 cells. The percentage of inhibition of DNA synthesis and colony formation after treatment with 15 μM genistein and low doses of radiation is reported to compare the data shown in Figs. 2–4; bars, SE.

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5 G. G. Hillman, personal communication.
This effect was dependent on continuous exposure of the cells to genistein.

We have demonstrated previously that genistein caused the PC-3 cells to arrest at the G2-M phase of the cell cycle (10). Ongoing studies on the mechanism of PC-3 cell killing by photons and neutrons in our laboratory show that radiation also causes accumulation of cells in the G2-M phase of the cell cycle, but this effect was more pronounced with photons than neutrons. Whether the combination of genistein with radiation causes a greater accumulation of cells in G2-M phase remains to be clarified. The molecular events leading to G2-M arrest may be the same or may differ for each modality alone and the combined modalities. The interaction of genistein and radiation may lead to new alterations in earlier events of the cell cycle, which ultimately will result in cell death. For example, the investigation of the effects of these two modalities on the transcription factor NF-kB may be warranted based on our previous observations that genistein can inhibit NF-kB DNA binding in PC-3 and LNCaP human prostate carcinoma cells (30). Moreover, genistein inhibited NF-kB activation by DNA-damaging agents, including H2O2, and tumor necrosis factor-α, resulting in cell apoptosis in these cell lines (30). In contrast to genistein, ionizing radiation was shown to activate NF-kB as an immediate-early response that functions to protect cells from apoptosis, a mechanism that could be implicated in cell survival to radiation, although it is still controversial (31, 32). We hypothesize that pretreatment of the cells with genistein may block NF-kB activation by radiation, inducing a cascade of molecular events driving the cells to an apoptotic pathway and, thus, increasing cell killing as shown in the present study. Further studies are under way to investigate the mechanism of interaction between genistein and radiation, both at the cellular and molecular levels by studying the genes involved in cell killing.

It should be noted that concentrations of genistein in the range of 5–15 μM are considered as mild doses and have shown no toxic effect on normal breast epithelial cells in vitro.6 In vivo, in human volunteers consuming 50 mg of isoflavone consisting of 40 mg of genistein and daidzein, the serum level of genistein was in the range of 15 μM, and no apparent toxicity was observed.7 Other studies have reported measurements of physiological concentrations of genistein in the range of 276 nm to 6 μM, depending on the study subjects and the isoflavone source (33, 34). Also, the doses of photon (200–300 cGy) or neutron (100–150 cGy) radiation, showing an optimal augmentation of cell killing when combined with 15 μM genistein, are low and nontoxic doses of radiation. These findings suggest that genistein may potentiate the effect of radiation therapy for localized prostate carcinoma and can be translated into the design of clinical protocols for prostate cancer patients with locally advanced disease to improve the outcome of radiation therapy.

REFERENCES


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6 F. H. Sarkar, personal communication.
7 Unpublished data.
Genistein Potentiates the Radiation Effect on Prostate Carcinoma Cells

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