Genistein Chemoprevention: Timing and Mechanisms of Action in Murine Mammary and Prostate\textsuperscript{1,2}


*Department of Pharmacology and Toxicology, †University of Alabama at Birmingham Comprehensive Cancer Center, and the **Department of Genomics and Pathology, University of Alabama at Birmingham, Birmingham, AL 35294

ABSTRACT We investigated the potential of genistein, the primary isoflavone of soy, to protect against breast and prostate cancers in animal models. For mammary cancer studies, Sprague-Dawley rats were fed AIN-76A diet \pm 250 mg genistein/kg diet. Dimethylbenz[a]anthracene was administered by gavage at d 50 postpartum to induce mammary tumors. Mammary cancer chemoprevention was demonstrated after prepubertal and combined prepubertal and adult genistein treatments but not after prenatal- or adult-only treatments, demonstrating that the timing of exposure to genistein is important for mammary cancer chemoprevention. The cellular mechanism of action was found to be mammary gland and cell differentiation, as shown by whole-mount analysis and \beta-casein expression. An imprinting effect was shown for epidermal growth factor receptor expression in mammary terminal end buds. For prostate cancer studies, we used two models. The first was a chemically (N-methylnitrosourea) induced prostate cancer rat model. Genistein in the diet inhibited the development of invasive adenocarcinomas in a dose-dependent manner. The second model was a transgenic mouse model that resulted in spontaneously developing adenosarcoma tumor of the prostate. Genistein in the diet reduced the incidence of poorly differentiated prostatic adenocarcinomas in a dose-dependent manner and down-regulated androgen receptor, estrogen receptor-\alpha, progesterone receptor, epidermal growth factor receptor, insulin-like growth factor-1, and extracellular signal-regulated kinase-1 but not estrogen receptor-\beta and transforming growth factor-\alpha mRNA expressions. We conclude that dietary genistein protects against mammary and prostate cancers by regulating specific sex steroid receptors and growth factor signaling pathways. J. Nutr. 132: 552S–558S, 2002.

KEY WORDS: • genistein • chemoprevention • mammary • prostate • cancer

Cancer is usually treated at the time of diagnosis, and chemoprevention is not usually considered until adulthood. However, because perinatal exposure to hormones and xenobiotics influences breast and prostate development and cancer, we have hypothesized that exposure to hormonally active nutritional chemicals during early windows of development plays a key role for cancer causation and prevention in these tissues.

The most convincing evidence indicating that environmental agents and early periods of development predispose for breast cancer is radiation exposure. Women exposed as teenagers to ionizing radiation are more susceptible for breast cancer than those exposed as adults (1,2). Moreover, early pregnancy or early exposure to the hormones of pregnancy reduces the incidence of breast/mammary cancer in women and animal models (3–5). This demonstrates that the early period of a woman’s life is crucial for predisposition to or for protection against breast cancer.

Asian women consuming a diet high in soy products have a low incidence of breast cancer (6,7), yet Asians who immigrate to the United States and adopt a Western diet lose this protection. Soy-based diets are high in phytochemicals and quantitative results indicate that isoflavone phytoestrogens are normal constituents of human urine from subjects consuming large amounts of soy products (tofu, soy flour, soy milk, tempeh, etc.) (8). Genistein is the predominant isoflavone phytoestrogen found in soy.
Genistein is a planar molecule with an aromatic A-ring, has a second oxygen atom 11.5 Å from the one in the A ring, and has a molecular weight similar to those of the steroidal estrogens (Fig. 1). It has estrogenic properties in receptor binding assays (9,10), cell culture (11,12), and uterine weight assays (13–15). Genistein inhibits topoisomerase II (16), platelet-activating factor- and epidermal growth factor-induced expression of c-fos (17), diacylglycerol synthesis (18), and tyrosine kinases (19). It also inhibits microsomal lipid peroxidation (20) and angiogenesis (21). Genistein exhibits antioxidant properties (22–24) and was reported to induce differentiation of numerous cell types (25–27). Most of these mechanistic data were derived from in vitro studies.

Genistein and mammary cancer

To investigate the potential of perinatal genistein exposure to protect against chemically induced mammary cancer, female Sprague-Dawley rats were fed 0, 25 and 250 mg genistein/kg AIN-76A diet starting 2 wk before breeding (28). Animal care and treatments were conducted in accordance with established guidelines and protocols approved by the University of Alabama at Birmingham Animal Care Committee. The dietary concentrations were chosen because they yield serum genistein concentrations in rats similar to blood genistein concentrations in men and women eating a diet high in soy (28,29). After parturition, dams and offspring were fed the same diets until time of weaning (d 21). From that time onward, all female offspring from the three treatment groups were fed AIN-76A diet only. At d 50 postpartum, dimethylbenz[a]anthracene (DMBA) (80 mg/kg body) was administered by gavage to induce mammary tumors. Animals were palpated for tumors and necropsied at 180 d after DMBA treatment or when tumors developed to 2.5 cm in diameter. Control animals (zero genistein, DMBA) developed approximately nine tumors, whereas dietary genistein suppressed DMBA-induced mammary tumor development in a dose-dependent manner. Rats exposed to 25 and 250 mg genistein/kg AIN-76A diets had 7.1 and 4.3 mammary tumors, respectively (Fig. 2). These data confirm our previous prenatal genistein study using a lower dose of DMBA (40 mg/kg body) (29). Data from Figures 2 and 3 show that the critical window for genistein chemoprevention is the postnatal period of the perinatal period.

Abbreviations used: DMBA, dimethylbenz[a]anthracene; EGF, epidermal growth factor; ER, estrogen receptor; MNU, methyl nitrosourea; TRAMP, transgenic mouse prostate adenocarcinoma.

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FIGURE 1 Structure of genistein.

FIGURE 2 Ontogeny of palpable mammary tumors in female Sprague-Dawley CD rats exposed perinatally to genistein in the diet. Starting at time of breeding, dams were fed 0, 25 and 250 mg genistein/kg AIN-76A diet. After weaning, the offspring were fed AIN-76A diet only. On d 50 postpartum, female offspring were treated with 80 mg dimethylbenz[a]anthracene (DMBA)/kg body. [Modified from (28). Permission requested from Oxford University Press.]

FIGURE 3 Ontogeny of palpable mammary tumors in female offspring of Sprague-Dawley CD rats fed genistein in the diet during pregnancy. Two groups of pregnant female rats (25 each) were fed 0 or 250 mg genistein/kg AIN-76A diet. At parturition both groups were fed AIN-76A diet until the time of necropsy (230 d postpartum). All offspring were treated with 80 mg DMBA/kg body on d 50 postpartum.
Our data (Fig. 3) demonstrate that dietary genistein administered prenatally did not alter predisposition for mammary cancer. In contrast, Hilakivi-Clarke et al. (33) reported that injecting pregnant rats with genistein resulted in increased susceptibility of the offspring for mammary cancer. We speculated that this apparent contradiction might be due to different routes of administration and bioavailability in the two studies. Circulating genistein concentrations from 21-d fetal, 7-d neonatal and 21-d prepubertal rats exposed to 250 mg genistein/kg AIN-76A diet were determined to be 43, 726 and 1810 nmol/L, respectively (28,29). This demonstrates genistein bioavailability during postnatal life but poor bioavailability prenatally. Also, we determined that ~46% of circulating total genistein is free genistein 24 h after injection of rats (34). This is in contrast to <2% being free (aglycone) genistein from dietary administration (28). The bioavailability of injected genistein is substantially greater than that of oral genistein (23-fold). Hence, we conclude that route of administration and timing of exposure determines the metabolism, bioavailability and biological action of genistein.

Because breast cancer has been demonstrated to be estrogen-dependent, we have been concerned that genistein, a phytoestrogen, may contribute to mammary cancer development. More specifically, women who have been diagnosed with breast cancer inquire whether soy products, including genistein, will protect from or cause a recurrence of their cancer. We attempted to address this in a laboratory study. Rats were fed AIN-76A diet ± 250 mg genistein/kg diet at three periods, and all females were treated intragastrically with 80 mg DMBA/kg body at d 50 postpartum. As seen in Figure 4, rats exposed to the control diet, AIN-76A only, from birth until the end of the experiment (Zero/DMBA/Zero) had the highest average number of tumors (9.0 tumors/rat). Rats exposed to genistein from d 1 to 21 postpartum only (Gen/DMBA/Zero) developed 4.5 tumors, which confirms our earlier work (28). Animals exposed to genistein from d 1 to 21 and 100 to 180 (Gen/DMBA/Gen) developed the fewest number of tumors (2.8 tumors/rat). The latter genistein feeding was initiated 50 d after the DMBA treatment, the time of onset of palpable mammary tumors. This demonstrates that genistein fed to adult rats previously exposed prepubertally to genistein provided these rats with additional protection against mammary cancer. Prepubertal genistein exposure seems to permanently affect the animal or mammary gland in a way that determines how that animal later responds to the same or similar chemical stimuli. In this case, genistein fed during the prepubertal period programmed future (adult) genistein response against mammary cancer susceptibility.

Table 1 summarizes the relationship among dietary genistein, timing of exposure and chemically induced mammary cancer in rats. Limiting exposure to dietary genistein to the prenatal or adult periods does not predispose or protect against mammary cancer. In contrast, exposure to dietary genistein during the prepubertal and prepubertal-plus-adult periods protected against chemically induced mammary cancer in rats. An epidemiological report using the Shanghai Cancer Registry, a case-control study, has shown an inverse relationship (50%) between adolescent (13–15 y old) soyfood intake and breast cancer incidence later in life (35).

**Genistein mechanism of action in the mammary gland**

Analysis of mammary gland morphology in rats treated with genistein revealed that its cellular mechanism of action is enhancement of mammary gland differentiation (28–31). We demonstrated that genistein administered to prepubertal rats reduced the number of terminal end buds and increased the number of lobules. Mammary terminal end buds are terminal ductal structures found primarily in young animals (and humans) and contain many undifferentiated epithelial cells (36,37). Terminal end buds are the most susceptible structures to chemical carcinogens; lobules are the terminal ductal structures most differentiated and least susceptible to chemical carcinogens.

Further evidence that genistein enhances differentiation was obtained by measuring β-casein in mammary glands. β-casein is a milk protein and biomarker of mature mammary glands and differentiated cells. Using Western blot analysis, we found that prepubertal genistein treatment increased β-casein expression in mammary glands of prepubertal and adult rats (Fig. 5). In the adult rats, β-casein was measured 30 d after genistein treatment.

One of the reasons cancer researchers have investigated

**TABLE 1**

*Dietary genistein, timing of exposure and mammary cancer chemoprevention*

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>Relative mammary tumor multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No genistein</td>
<td>8.9</td>
</tr>
<tr>
<td>Prenatal genistein</td>
<td>8.8</td>
</tr>
<tr>
<td>Adult genistein (after tumors)</td>
<td>8.2</td>
</tr>
<tr>
<td>Prepubertal genistein</td>
<td>4.3</td>
</tr>
<tr>
<td>Prepubertal and adult genistein</td>
<td>2.8</td>
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Diets contained ± 250 mg genistein/kg AIN-76A.

1 All rats were treated with 80 mg dimethylbenz[a]anthracene/kg body weight at d 50 postpartum.
2 Prenatal treatment is throughout gestation.
3 Adult treatment was initiated at 100 d postpartum.
4 Prepubertal treatment was from d 1 to 21 postpartum.
genistein as a chemopreventive agent is the reports that it inhibits protein tyrosine kinases in vitro (18,19). As an extension of this, we investigated the potential of genistein to regulate the epidermal growth factor (EGF) receptor in vivo. In 21-d-old rats treated prepubertally with genistein, we found increased EGF receptor expression in mammary terminal end buds (38). Not only was this finding contrary to the in vitro reports, but this was surprising because we expected a chemopreventive agent to down-regulate the expression of this growth factor signaling pathway. However, when we extended our studies to 50-d-old rats treated prepubertally with genistein, we observed that EGF receptor expression was down-regulated in terminal end buds of these rats. We interpreted this to mean that early in postnatal life, genistein initially up-regulated the EGF-signaling pathway to enhance mammary gland development that resulted in early mammary gland differentiation. Reduced EGF signaling and decreased cell proliferation at 50 d when the DMBA was given was associated with reduced susceptibility to chemical carcinogenesis (28–31,36,37). Developmental modifications by a hormonally active chemical that result in altered biochemical or behavioral responses later in life was defined as imprinting (39–41). We speculate that down-regulated EGF receptor signaling in mammary terminal end buds at the time of carcinogen exposure plays a role in reduced mammary cancer development.

Genistein and mammary cancer chemoprevention summary

We have demonstrated that prepubertal and prepubertal-plus-adult genistein exposures protect against chemically induced mammary cancer in rats. We conclude that for genistein to protect against breast cancer, initial exposure must occur during the early sensitive period of mammary gland development, that is, the neonatal through prepubertal periods. The cellular mechanism of action of genistein is to enhance mammary cell differentiation (28–31). One identified biochemical mechanism is short-term and direct up-regulation of the EGF-signaling pathway that plays a role in cell differentiation (38). Paradoxically, this results in the epithelial cells of the mammary terminal end buds of adult animals having reduced EGF receptor expression. EGF signaling has been associated with cell proliferation; hence, we believe that down-regulated EGF receptor in adults contributes to the genistein chemoprevention. In reference to genistein in adults conferring additional protection when given to rats previously exposed to genistein compared with genistein-naive animals, we speculate that the early developmental effects have altered the molecular blueprint from which mammary cells respond to cancer initiators and promoters. Our laboratory data are consistent with the epidemiological report showing an inverse relationship between adolescent soyfood intake and breast cancer incidence later in life (35). We conclude that the most sensitive period for mammary cancer chemoprevention in the rat is the prepubertal period and in the human is probably the adolescent period.

Genistein and prostate cancer

Prostate cancer is the second leading cause of cancer death in men. Epidemiological data indicate that the incidence and mortality of prostate cancer are considerably lower in Asian populations than in U.S. and European populations (42), yet the incidence of precancerous lesions is the same for these populations (43). Upon emigration to the United States, Asian men have a greater risk for developing prostate cancer, and the earlier in life their arrival, the more closely their risk approaches that of American men (44).

One of the major differences between Asian and Western populations is diet. Asians have traditionally consumed a soy-based diet containing isoflavones, resulting in higher genistein concentrations in the blood and urine than those of American men (45,46). Our goal was to investigate the potential of genistein in the diet to protect against prostate cancer.

For the first prostate chemoprevention study, Lobund-Wistar rats were exposed to 0, 25 and 250 mg genistein/kg AIN-76A diet starting at conception and continuing until necropsy at age 11 mo (47). From d 50 to 66 postpartum, male offspring were given 33 mg flutamide/kg body daily by gavage to cause chemical castration. On d 67, 68 and 69, they were injected daily with 25 mg testosterone/kg body to stimulate mitosis. On d 70, all rats were anesthetized and 42 mg N-methylnitrosourea (MNU)/kg body was injected into the dorsal prostate for cancer initiation. One week after MNU administration, silastic implants of 25 mg testosterone were administered (and replaced every 12 wk) to stimulate mitosis and promote tumor growth. By age 40 wk, palpable prostate tumors were detectable. Rats were necropsied when 48 wk old or when they became moribund. In rats with small tumors, the tumors were confined to the site of MNU injection, demonstrating target organ specificity.

Rats fed the control diet, AIN-76A, and subjected to the carcinogenesis protocol developed 86.4% incidence of prostate tumors by 11 mo old (Fig. 6). Rats exposed to 25 and 250 mg genistein/kg diet had tumor incidences of 77.8% and 63.0%, respectively. The percentage of prostate tumors that were classified as invasive adenocarcinomas in rats fed 0, 25 and 250 mg genistein/kg diet were 77.3%, 61.1% and 44.4%, respectively. This was a dose-dependent significant decrease in prostate adenocarcinoma development (47). We conclude that lifetime dietary genistein protected against chemically induced prostate cancer development in rats.

The second model used for investigating genistein chemoprevention of prostate cancer was a transgenic mouse model that spontaneously develops prostate cancer, transgenic mouse
prostate adenocarcinoma (TRAMP) (48). The TRAMP mouse was developed by using the prostate-specific probasin promoter to drive expression of the simian virus 40 early gene in the prostatic epithelium. The SV40 T antigen (Tag) acts as an oncprotein through interactions with the p53 and retinoblastoma tumor-suppressor gene products. All TRAMP mice develop changes resembling human prostate intraepithelial neoplasia and poorly differentiated tumors, ultimately developing prostatic adenocarcinomas that metastasize to distant sites, primarily the lymph nodes, bone and lungs (49,50).

In our experiments (51), approximately one-half of the transgenic male mice displayed well-differentiated prostatic adenocarcinomas that metastasize to distant sites, primarily the lymph nodes, bone and lungs (49,50).

At necropsy, serum genistein concentration was determined and selected organs were weighed and prepared for histopathological evaluation (51). Serum genistein concentrations in mice on diets containing 0, 250 or 500 mg genistein/kg AIN-76A were 52 ± 33, 139 ± 70 and 397 ± 105 nmol/L, respectively, comparable with those found in Asian men on a regular soy diet (276 nmol/L) (45). As indicated by body and organ weights, dietary genistein had no toxic effect on TRAMP mice.

Sex steroid and growth factor signaling in the prostate

The interaction of sex steroid and growth factor signaling pathways is believed to be critical in the process of development and differentiation of hormone-responsive tissues and for cancer in the prostate (52). However, whether steroid hormones mediate the effects of growth factors or vice versa is unclear. Sex steroid-induced epithelial cell proliferation and differentiation have been associated with the coordinated induction of several peptide growth factors and their receptors, including some that are tyrosine kinase-dependent.

Nontransgenic and TRAMP mice were fed AIN-76A diet until 6 wk old, when one group of 14 TRAMP mice was fed 250 mg genistein/kg diet. An equal number of TRAMP and nontransgenic mice were fed AIN-76A diet only. At 12 wk old, the three groups of mice were killed and the dorsolateral prostate of TRAMP mice but before development of adenocarcinoma tumor (48,53,54). RNA was isolated and reverse-transcribed and the cDNA was amplified by polymerase chain reaction. Relative quantitative differences in cDNA were determined from data obtained during the exponential phase of amplification. In comparing prostates of transgenic and nontransgenic mice, we observed that androgen receptor, estrogen receptors (ER-α and -β), progesterone receptor, EGF receptor, transforming growth factor-α, insulin-like growth factor 1 and extracellular regulating kinase-1 mRNA transcripts were significantly higher in the transgenic mice (C. A. Lamantiniere and J. Wang, unpublished data, 2001). We speculate that increased sex steroid and growth factor signaling contribute to the increased incidence of spontaneously developing prostate cancer in transgenic mice.

In contrast, the prostates of transgenic mice fed the genistein-containing diet had reduced androgen receptor, ER-α, progesterone receptor, EGF-receptor, insulin-like growth factor I and extracellular regulating kinase-1 mRNA transcripts compared with prostates from TRAMP mice fed a
diet devoid of genistein (C. A. Lamantiniere and J. Wang, unpublished data, 2001). ER-β and transforming growth factor-α mRNA were not altered by genistein. We speculate that genistein down-regulates expression of specific proteins and regulates cell proliferation and prostate cancer development. Should this down-regulation be extended to these sex steroid receptor and growth factor ligand and receptor proteins, this could provide a biochemical mechanism for the suppression of prostate cancer by genistein. Most interesting is that ER-β was not modulated by genistein. Not only does genistein bind with a greater affinity to ER-β than to ER-α (55), but the two ER have been shown to signal in different ways depending on ligand and response element. Also, ER-β is more involved in cell differentiation and ER-α is more involved in cell proliferation (56). Selective actions by genistein could explain both prostate gland differentiation via ER-β activation and reduced cell proliferation via down-regulated ER-α expression.

**Genistein and prostate cancer summary**

We demonstrated, in two animal models, that dietary physiological amounts of genistein can protect against chemically induced and spontaneously developing prostate cancers (47,51). We presented evidence that dietary genistein regulates, with specificity, sex steroid receptor and growth factor ligand and receptor mRNA expression. We speculate that these gene products contribute to chemoprevention of prostate cancer by genistein. Because postpubertal genistein exposure protects against prostate cancer development and can regulate sex steroid and growth factor signaling in animal models, we believe that genistein (or soy) can protect against prostate cancer in men.

**DISCUSSION**

We have demonstrated that the primary isoflavone component of soy, genistein, can protect against mammary (28–31) and prostate (47,51) cancers in rodent models. For mammary cancer protection, genistein exposure must first occur early in postnatal life. The importance of early mammary gland differentiation and carcinogenesis probably lies in pubertal development and estrogen surge, leading to oxidative DNA damage and cancer initiation. If the epithelial cells of the terminal end buds are differentiated, they are less susceptible for cancer (36,37). Also, we have demonstrated that early exposure to genistein exerts an imprinting-like effect on EGF receptor expression (38), a signaling pathway that plays a significant role in cell proliferation and cancer ontogeny. Imprinting is considered to set the pattern of gene expression early in development from which the adult responds; the pattern includes regulation of steroid receptor mechanisms and signal transduction. Consistent with this is the present demonstration that offspring imprinted early in life gain additional breast cancer protection with adult dietary exposure. Furthermore, we speculate that mammary cancer chemoprevention via cell differentiation and imprinting is not restricted to genistein. We believe that women imprinted by other means, for example, pregnancy or other nutritional differentiating chemicals, could benefit from ingesting soy as adults.

In reference to prostate cancer, we demonstrated that dietary genistein initiated at puberty suppressed spontaneously developing prostate cancer in transgenic mice (51). Short-term feeding of genistein from the pubertal to young adult period was able to down-regulate specific sex steroid receptor and growth factor ligand and receptor mRNA expression. We demonstrated that it is not necessary to give pharmacologic concentrations of genistein to get beneficial effects. TRAMP mice fed genistein had serum genistein concentrations (125–400 nmol/L) (51) comparable with blood genistein concentrations of Asians eating a traditional diet high in soy (276 nmol/L) (45,46). This supports our earlier report that dietary physiological amounts of genistein could regulate biochemical actions in the prostate, that is, EGF receptor expression in rats (57).

**LITERATURE CITED**


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