Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein

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The estrogenic soy isoflavone, genistein, stimulates growth of estrogen-dependent human breast cancer (MCF-7) cells in vivo. Genistin is the glycoside form of genistein and the predominant form found in plants. It is generally believed that genistin is metabolized to the aglycone genistein in the lower gut. However, it is unclear if the rate of metabolism of genistin to genistein is sufficient to produce a level of genistein capable of stimulating estrogen-dependent breast cancer cell growth. Our hypothesis was that dietary genistin would stimulate tumor growth similar to that observed with genistein in athymic mice. To test this hypothesis, genistin or genistein was fed to athymic mice containing xenografted estrogen-dependent breast tumors (MCF-7). Mice were fed either genistin at 750 p.p.m. (parts per million) or genistein at 1200 p.p.m., which provides equal molar concentrations of aglycone equivalents in both diets. Tumor size was measured weekly for 11 weeks. At completion of the study, half of the animals per treatment group were killed and tumors collected for evaluation of cellular proliferation and estrogen-responsive pS2 gene expression. Incorporation of bromo-deoxyuridine into cellular DNA was utilized as an indicator of cellular proliferation. Dietary genistin resulted in increased tumor growth, pS2 expression and cellular proliferation similar to that observed with genistein. The remaining mice were switched to diets free of genistin and genistein. When mice were placed on isoflavone free diets, tumors regressed over a span of 9 weeks. Next, we examined how effectively and where metabolism of genistin to genistein occurred in the digestive tract. We present evidence that demonstrates conversion of genistin to its aglycone form genistein begins in the mouth and then continues in the small intestine. Both human saliva and the intestinal cell-free extract from mice converted genistin to genistein. In summary, the glycoside genistin, like the aglycone genistein, can stimulate estrogen-dependent breast cancer cell growth in vivo. Removal of genistin or genistein from the diet caused tumors to regress.

Introduction

Isoflavones are a group of compounds derived from plant sources such as soy and other legumes that are being consumed in the diet for beneficial effects related to health. Genistein is the most studied of the isoflavones and has been shown to have multiple biological activities including inhibition of topoisomerase II activity (1,2) and inhibition of tyrosine kinase activity (3,4) resulting in cancer cell death. However, these studies have been conducted in vitro and require concentrations >10 µM before effects are observed. Plasma concentration of genistein in humans after consuming a meal containing soy is 2–4 µM and most of this is in the glucuronide conjugated form (5). It is unlikely that the concentrations needed to achieve the biological effects observed in vitro can be reached with normal dietary consumption of the isoflavones. On the contrary, at concentrations as low as 200 nM genistein has been demonstrated to act as an estrogen agonist and promote the growth of estrogen-dependent human breast cancer (MCF-7) cells in vitro. In these cells, an estrogen-responsive gene, pS2, is upregulated suggesting that the stimulation of cellular growth is a result of estrogenic activity of genistein (6,7). Further, the ability of dietary genistein to stimulate the growth of MCF-7 cells in vivo has been shown when fed to ovariectomized athymic nude mice transplanted with MCF-7 cells. In this study, ovariectomized mice consuming genistein at the concentration of 750 p.p.m. in their diet had increased number and size of the end buds within the mammary gland (7). These data suggest that genistein has the ability to act as an estrogen agonist in the mammary gland of mice and can stimulate the growth of estrogen-dependent breast tumors.

While there has been extensive investigation into the biological activity of isoflavones, it is important to note that the predominant form of these compounds in plants as well as in many of the dietary supplements is as their glycoside conjugates. The glycoside forms of these molecules consist of the isoflavone covalently bound to a glucose molecule. Studies in the 1970s revealed that 99% of the isoflavonoid compounds in soy are present as their glycosides (8). Genistin is the glycoside form of genistein. It is generally believed that genistin is hydrolyzed removing the covalently bound glucose to form genistein and that genistein is the form of the compound that is absorbed in the intestine and is the form responsible for the biological activities of the isoflavone. The conversion of genistin to the aglycone, genistein, is important because the majority of the isoflavone consumed is in the glycoside form. It was first demonstrated that the gut microflora played a role in the conversion of genistin to genistein, when rumen bacteria were shown to produce β-glucosidases capable of hydrolyzing dietary glucosides to produce aglycones (9,10). More recently, it has been demonstrated that enzymes present in the human small intestine and liver also have the ability to convert genistin to genistein (11). Thus, the conversion of genistin to genistein begins prior to the compound reaching the microflora of the intestine. However, it was observed that men consuming soy flour in a single serving have measurable plasma concentrations of genistein within 30 min after ingestion (12). The time required for a food bolus containing genistin to travel to the small intestine and for conversion to occur would likely take longer than the 30 min after which genistein is observed in
the blood. These data suggest that some conversion and absorption of the compound must be occurring in the digestive process prior to the small intestine.

The research presented here was designed to address two objectives. First, to test the hypothesis that dietary treatment of genistin in mice would cause stimulation of growth of human estrogen-dependent breast cancer cells transplanted into athymic mice in a manner similar to that seen with the aglycone form, genistein. Second, to investigate the role that human saliva plays in the conversion of genistin to genistein.

Materials and methods

Effects of consumption of genistein and genistin on the growth of estrogen-dependent tumors

Animals. Female athymic nude mice were purchased from Harlan Sprague Dawley (Indianapolis, IN) and delivered at 28 days of age. Mice were ovarioctomized at 21 days of age and allowed 1 week to recover prior to delivery. A 2 mg 17β-estradiol (E2) pellet was placed under the skin of each mouse before MCF-7 cells were transplanted into the animal. Cells (1×10^6 cells/site) were then injected into the four flanks on the back of each animal. Within the first 4 weeks, tumors that subsequently formed were allowed to develop to an average cross-sectional area of ~40 mm^2. At this point, animals were randomly assigned to one of four treatment groups (12 animals/group). The treatment groups were: (i) positive control; (ii) negative control; (iii) dietary genistein 1200 p.p.m.; and (iv) dietary genistin 1200 p.p.m. The E2 pellet was removed from the negative control animals and from all of the mice on the various dietary treatments. The E2 pellet was also removed from the positive control group, but these animals were again implanted with an identical E2 pellet. Negative and positive controls were given American Institute of Nutrition 93 growth diet (AIN 93G) as a control diet. The remaining animals were put on one of the two treatment diets which were either AIN 93G supplemented with either genistein or genistin at the concentrations stated above. Tumor area and body weight were measured weekly. At the end of the study, tumors and plasma samples were collected for analysis.

Diet formulation. AIN 93G semipurified diet was selected as a basal diet for control animals as it has been established as meeting all of the nutritional requirements of mice (13). Animals in two treatment groups were fed either AIN 93G diet plus genistein or AIN 93G plus genistin. Genistein was provided at the final concentration of 750 μg/p.p.m. as our laboratory has demonstrated in the past that this level is sufficient for stimulating the growth of MCF-7 tumors in vivo (7). Genistin was then supplemented at a concentration that if completely hydrolyzed would provide an equal amount of genistin. This substitution resulted in a final concentration of 1200 p.p.m. genistin. Therefore, assuming complete conversion, both provided 750 μg/p.p.m. aglycone equivalents.

Estrogen pellet preparation. MCF-7 cells will not produce tumors in ovarioctomized mice unless they are supplemented with estrogen. Therefore, 1 week after delivery, animals were implanted with E2 pellets. Estradiol pellets were made containing 2 mg of 17β-estradiol mixed with 18 μg of cholesterol as a carrier. A 20 mg mixture containing E2 and cholesterol was placed into a pellet mold and pressed into a compact pellet ~4.5 mm in diameter and ~2.5 mm in depth. Pellets were then placed subcutaneously in the interscapular region of mice (14).

Tumor implantation. MCF-7 cells were maintained in 100×20 mm polystyrene tissue culture plates in improved minimal essential medium (IMEM) (Biofluids) containing 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were grown to confluence, trypsinized, washed and counted. Cells were suspended in matrigel matrix (Becton Dickinson) before being injected (15) at 1×10^6 cells in (40 μl) per site into each of the four flanks of the athymic mice.

Tumors were measured weekly and cross-sectional area was determined using the formula [length / 2]×[width / 2]×π^3/6 (7,14,16). When tumors reached an average cross-sectional area of 40 mm^2, animals were randomly assigned into treatment groups with each group normalized for tumor number, tumor size and animal number. E2 pellets were removed from all animals and the mice were then placed on the treatment diets. Positive control mice were reimplanted with a fresh pellet containing 2 mg E2. We then resumed measuring tumor areas and body weights weekly.

Tissue/tumor collection. At the completion of the study, mice were killed by cervical dislocation and tumors were harvested. Tumors from each mouse were then weighed, 10% formalin added and embedded for immunohistochemical staining. Additional tumors from each mouse were immediately frozen by immersion in liquid nitrogen for RNA isolation and analysis.

Analysis of estrogen-responsive pS2 mRNA

RNA preparation. The mRNA was isolated using procedures routinely used in our laboratory (17). Briefly, frozen tumors (~200 mg) from liquid nitrogen were smashed and the coarse tumor powder was transferred into TRIZOL® (Gibco BRL, Grand Island, NY) in a 15 ml tube and was homogenized using a Polytron-Aggregate (Luzern, Switzerland). Chloroform was added into a homogenized tumor sample, shaken vigorously, and then incubated for 10 min at 24 °C. The reaction tube was centrifuged at 12 000 g for 15 min at 4 °C. The RNA pellet was washed with ice-cold 75% ethanol and centrifuged at 7500 g for 5 min at 4 °C. The RNA pellet was air-dried, then dissolved into RNase-free deH2O. RNA was stored at –80 °C. RNA concentrations were measured at 260 and 280 nm (1 OD260 = 40 μg of single stranded RNA/ml).

Northern blot analysis. Expression of pS2 was utilized as a biomarker of estrogenic activity (18). For the detection of pS2 expression, 10 μg of RNA were separated on 1.2% formaldehyde denaturing agarose gels and transferred to a Magnacharge, Nylon, Transfer membrane (Osmonics, Westboro, MA). The RNA was UV cross-linked onto the membrane. The membrane was prehybridized in a formamide pre-hybridization solution containing denatured salmon sperm DNA for 3 h at 42 °C. After 3-h pre-hybridization, the DNA probe was labeled using Random Primers DNA Labeling System (Gibco BRL). For the estrogen-responsive pS2 gene, 25 ng of pS2 cDNA, or for the control 25 ng of GAPDH cDNA probe was labeled with 50 μCi [α-32P]dCTP. The membrane was incubated with 32P-labeled probe overnight at 42 °C. The membrane was washed twice with 5 ml of 0.2% SSC/0.1% SDS at 24 °C for 5 min each, three times with 5 ml of 0.1% SSC/0.1% SDS at 68 °C for 15 min each, and rinsed with 2% SSC. The membrane was exposed to X-ray film for 6 h, and hybridizing RNA molecules were detected by performing autoradiography. Film was analyzed using Collage™ (Version 4.0) (Image Dynamics) with Foto Analyst (Fotodyne, Hartland, WI).

Tumor cell proliferation immunohistochemistry

BrDU (5-bromo-2'-deoxyuridine) analysis. BrDU incorporation into cellular DNA was used as an indicator of cells that were actively proliferating (19). Four hours prior to killing the animals, each mouse was injected intraperitoneally with 50 μg BrDU/kg body weight. Tumors were excised, skin and fat removed, and processed. Processing included a fixation period of 24 h in 10% formalin followed by paraffin embedding of the tissues. Then, 3–5 μM sections were cut from each tumor. Prepared sections were then stained for the presence of BrDU utilizing a modified immunohistochemistry protocol (20). Briefly, slides were deparaffinized and hydrated by immersing in xylene twice for 12 min and immersing in a series of alcohol/water solutions for 5 min each. To block endogenous peroxidase, slides were immersed in 0.3% H2O2 for 20 min then washed with distilled water. Slides were then microwaved in a Pyrex dish in citrate buffer at pH 6 for 20 min and cooled. Then slides were washed in phosphate-buffered saline (PBS) (pH 7.1–7.4) for 5 min and tissue sections were rimmed with wax. Fifty microliters of antiBrDU primary antibody (Amersham, Piscataway, NJ) was added to slides and incubated for 1 h at 24 °C in a humidity chamber. Slides were washed with PBS and 1 ml of diluted secondary antibody (DAB) plus Ni enhancer solution was added to each slide. Slides were then washed in water twice, in PBS, and counterstained with 2% hematoxlin for 1 min. The slides were then dehydrated by placing them in 80% ethanol for 5 min, 95% ethanol for 5 min, and 100% ethanol for 5 min followed by xylene four times for 5 min each. Slides were then coverslipped and analyzed by light microscope. Both positive and background stained cells were counted in a given area of tissue. The data were then presented as percentage of cells proliferating in a given area of tumor.

Salivary and small intestinal cell-free extract metabolism of genistin to genistein

Tissue preparation and protein analysis. Both intestinal cell-free extract and salivary samples were used to analyze the ability of these tissues to convert previously given either dietary genistin or genistein were then placed on AIN 93G control (isoflavone free) diet. The remaining negative control animals were kept on the AIN 93G control diet. We then resumed measuring tumor areas and body weights weekly.
Genistin causes estrogen-dependent breast tumor growth

Results

Effect of genistin and genistein on MCF-7 tumor growth in athymic mice

Four weeks after re-treatment with a new 2 mg E₂ pellet, the average cross-sectional area of the tumors in the positive control group was 103 mm² (Figure 1). At this point, these mice were killed. Eleven weeks after the E₂ pellets were removed, the negative control tumors regressed to an average area of 25 mm². Both genistin and genistein stimulated the growth of MCF-7 tumors (Figure 1). By week 11 after E₂ pellet removal, tumors from both the genistin and genistein treatment groups were significantly (P < 0.01) larger than that of the negative control group with average tumor areas of 85 and 74 mm², respectively. The average tumor areas in the genistin and genistein-treated groups were not significantly different from each other. Eleven weeks after re-treatment with a new E₂ pellet, half of the animals were killed for tissue collection. The remaining animals of the negative control, genistin and genistein-treated groups were then all given the AIN 93G control diet. The tumors in the negative control group continued to regress during this time resulting in a final average tumor area of 7 mm² (Figure 2). Nine weeks after removing genistin and genistein from the diet of treated animals, tumors in these groups regressed to average sizes of 15 and 17 mm², respectively. While the average tumor size was reduced 7-fold, the final measurements were still significantly (P < 0.05) different from the negative controls in both the genistin and genistein. However, because of the slope of the regression in tumors of both treatments, it can be estimated that the tumors would regress to a point where they were no longer significantly different than the negative controls (Figure 2).
with a new E2 pellet while tumors were collected from the positive control group 4 weeks after re-treatment from tumors in each treatment group. Tumors were collected we conducted northern blot analysis using mRNA isolated to enhance the expression of an estrogen-responsive, pS2 gene.

To evaluate the ability of genistin and genistein in the diet to affect estrogen-responsive, pS2 mRNA expression in MCF-7 tumors, treatment groups were positive control (PC) which were implanted with a 2 mg E2 pellet, negative control (NC) which had no E2 pellet or dietary isoflavone, dietary genistein (GEN) at 750 p.p.m. and dietary genistin (GIN) at 1200 p.p.m. At the end of the study tumors were collected for analysis. The animals consuming genistin and genistein had proliferation in tumors excised from animals consuming genistin and genistein.

**Cellular proliferation in MCF-7 tumors excised from animals consuming soy protein isolates and genistein**

Cellular incorporation of BrdU was utilized as an indicator of cellular proliferation in MCF-7 tumors. Cells that stained positive after immunohistochemical analysis were considered actively proliferating cells. Both proliferating and non-proliferating cells in a given field of view were counted and final values were expressed as percentage of proliferating cells. The percentage of proliferating cells value for the negative control group was 1.4%. Positive control animals remaining on estrogen supplementation had a significantly higher percentage of cellular proliferation when compared with the negative control group with a value of 12.6% proliferation. The animals consuming genistin and genistein had proliferation of 7.8 and 11.5%, respectively. These values were significantly higher than the negative controls and the proliferation in the genistein-treated animals was not significantly different than that of the positive control group (Figure 4).

**Effect of genistin and genistein on estrogen-responsive pS2 mRNA expression in MCF-7 tumors**

To evaluate the ability of genistin and genistein in the diet to enhance the expression of an estrogen-responsive, pS2, gene we conducted northern blot analysis using mRNA isolated from tumors in each treatment group. Tumors were collected from the positive control group 4 weeks after re-treatment with a new E2 pellet while tumors were collected from the negative control group with a value of 12.6% proliferation. The data are presented as the percentage of cells actively proliferating in a given area of tissue ± SEM.
Table I. Conversion of genistin to the aglycone form genistein by either saliva or small intestine cell-free extract samples

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Genistin conversion (% converted)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>12</td>
<td>70.0</td>
<td>± 4.4</td>
</tr>
<tr>
<td>Small intestine cell-free extract</td>
<td>22</td>
<td>58.5</td>
<td>± 3.2</td>
</tr>
</tbody>
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*For each reaction, either saliva or small intestinal cell-free extract was added at a volume that resulted in 200 μg protein. Genistin was then added to result in a final concentration of 30 μM genistin. A sample was taken from each reaction before and after a 90-min incubation at 37°C to analyze genistin levels. Percentage conversion was then calculated utilizing the equation (ng genistin after incubation/ng genistin before incubation)×100%.

†Saliva samples were collected from human volunteers and used immediately after collection.

‡Small intestine cell-free extracts were prepared from the small intestines of mice.

Conversion of genistin to the aglycone form genistein when exposed to saliva or small intestinal cell-free extract

The ability of either saliva or the small intestine to remove the glucose from genistin to form the aglycone form genistein was evaluated. To quantify this conversion, a sample was taken from each reaction to establish a starting concentration of genistin. Then after a 90 min incubation the remaining genistin was measured. The data are expressed as a percentage of genistin converted to genistein. Saliva collected from human volunteers resulted in a 70% conversion of genistin to its aglycone form. Small intestine cell-free extract samples collected from mice resulted in a conversion rate of 58.5% (Table 1).

Discussion

Dietary genistin was able to elicit an effect on the growth of estrogen-dependent tumors similar to that of genistein. This is of significance as the glycoside, genistin is the predominant form of the isoflavone found in soy food products and in dietary supplements used for medicinal purposes. It has been reported that isoflavone aglycones are absorbed faster and in greater amounts than their glycoside forms producing higher concentrations of total isoflavones in the plasma (21). However, in this study plasma levels of total genistein were similar in both the genistin and genistein fed animals with plasma concentrations of 0.51 and 0.44 μM, respectively (data not shown). These measurements are consistent with concentrations observed in previous studies where rodents were fed genistein (0.93 μM) (22) and are similar to levels measured in humans 24 h after consuming isoflavones from soy milk (0.53 μM) (23). In this study, the removal of either genistin or genistein diet resulted in a rapid regression of tumor size demonstrating a direct relationship between tumor growth and isoflavones in the diet. Tumor growth appeared to be a result of enhanced cellular proliferation as demonstrated by the immunohistochemistry data in which actively proliferating cells are preferentially stained. Both genistin and genistein increased the percentage of cells proliferating in these tumors resulting in the increased tumor size. An increase in the relative abundance of pS2 mRNA as observed from northern blot analysis confirms that both genistin and genistein can elicit an estrogenic response in the tumors. These data suggest that the increase in proliferation is mediated via an estrogen-dependent mechanism. Therefore, we have demonstrated that the estrogenic isoflavone genistein whether in its aglycone or glycoside form can stimulate the growth of estrogen-dependent human breast cancer tumors transplanted into athymic mice. These findings suggest possible adverse effects for women whom have breast cancer and are consuming genistein in their diet.

Breast cancer incidence increases with age and three out of four women with breast cancer are over 50 years of age. Therefore, post-menopausal women are at a higher risk of being diagnosed with breast cancer than younger pre-menopausal women (24). Approximately half of the breast cancer in these women will be estrogen-dependent. Introduction of an estrogen, whether through hormone replacement therapy or through supplementation with dietary isoflavones, has the potential for negative effects regarding the stimulation of the growth of these estrogen-dependent tumors. It was unclear whether dietary genistin could produce similar biological results to that observed with genistein. We present here that both the glycoside and aglycone forms of the isoflavone have the ability to stimulate the growth of MCF-7 tumors xenografted into athymic mice. It is important to note that only one human breast cancer cell line was used in this study and that tumor growth is observed in the mouse, which could vary somewhat from what occurs in humans. However, this model has been utilized in the development of drugs used to prevent and treat breast cancer such as tamoxifen and findings in this model are thought to be relative to what will occur in humans (25). It should also be mentioned that genistein has been demonstrated to be biologically active when consumed by humans in the diet. Menstrual cycles of women consuming textured vegetable protein containing 45 mg of isoflavones daily for a month were prolonged by 2 days as a result of a longer follicular phase (26). Similar lengthening of the menstrual cycle was observed in women consuming 60 g soy protein isolate containing 45 mg isoflavones daily for a month (27). Isoflavones were shown to act estrogenically within the human mammary gland when mammary duct fluid aspirates from women ingesting 38 g of soy protein isolate containing 38 mg genistein, contained a higher number of hyperplastic epithelial cells when compared with controls (28). A more recent study reported elevated nipple aspirate apolipoprotein D and pS2 expression in women consuming 60 g of textured vegetable protein for 2 weeks (29). The knowledge that dietary genistein and genistin can have a similar effect on growth of human estrogen-dependent tumors in the athymic transplant model raises the concern that self-medication by dietary supplementation of estrogenic isoflavones could result in increased risk in post-menopausal women with pre-existing estrogen-dependent tumors.

The second objective of this research was to evaluate the location of hydrolysis of genistin to genistein in the digestive tract. The isoflavones were first identified when a reduction of reproductive performance was reported in sheep grazing on subterranean clover (30) and genistein was revealed as one of the compounds responsible for this condition (31). Much of the original isoflavone research was conducted in ruminant species. It has been demonstrated that intestinal bacteria were capable of converting the glycosides to their aglycone forms (9,10). Following these findings it was widely believed that an intact gut microflora in the lower intestine of monogastrics was an absolute requirement for the normal metabolism of genistein. Day et al. (1998) demonstrated that there are other means in which the glycoside is converted. This study showed
the existence of enzymes produced by the small intestine as well as the liver that are capable of hydrolyzing the glycoside to the aglycone (11). Specifically, lactase phlorizin hydrolase, a membrane-bound β-glucosidase, found on the brush border of the mammalian small intestine has the ability to perform this hydrolysis (32). It is then likely that the resulting genistein is readily absorbed through the small intestine (33). Other studies confirm the ability of the small intestine to convert genistin to genistein. Utilizing rat small intestines in an ex vivo intestinal perfusion model, ~15% of the genistin administered appeared on the vascular side predominately as genistein glucuronide with some free genistein and minor amounts of unmetabolized genistin (34). When genistin was administered in the form of pre-digested tofu the majority of the genistin appeared vascularly as free genistein (35). The ability of the small intestine to convert genistin to genistein probably contributes to the absorption of genistein. However, it does not fully explain the observation that the aglycone forms of the isoflavones appear in the plasma within 30 min following ingestion of soy flour containing the glycoside forms of the compounds (12). While conversion and rapid absorption in the small intestine may partially explain this early appearance of plasma genistein it is probable that at least one other mechanism is involved in early absorption of the compound. Piskula et al. (1999) demonstrated that unlike in the small intestine, genistein but not genistin was readily absorbed through the wall of the stomach (36). These findings collectively suggest that at least some level of conversion likely occurs prior to food entering the stomach and that the resulting genistein could then be absorbed through the stomach wall. Here we report that human saliva is capable of converting genistin to genistein. The exact mechanism and relative importance of this conversion to the absorption of genistein is not known and will require further investigation. To date, the efficiency and physiological contribution of the conversion of genistin to genistein at each of these sites in the digestive tract are unclear. Data reported here indicate that metabolism of the glycoside genistin begins in the mouth rather than the small intestine.

In summary, dietary genistin is capable of eliciting biological activity similar to genistein with regards to stimulation of growth of estrogen-dependent breast cancer tumors transplanted into athymic mice. Further, the tumors appear to have an absolute requirement for the phytosterogens in the absence of high endogenous E2 levels as demonstrated by the regression of the tumors when genistin and genistein were removed from the diet. The stimulatory effect of genistin appears to be directly related to the estrogen agonist activity of the compound that results in an increase in cellular proliferation within the tumors. It is likely that the activity of genistin is a result of the efficient conversion and absorption of genistin to genistein, which is the biologically active form of the compound and that contrary to previous reports that this metabolism begins in the mouth upon ingestion of the glycoside form of the compound.

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References

Genistin causes estrogen-dependent breast tumor growth